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TERAMO

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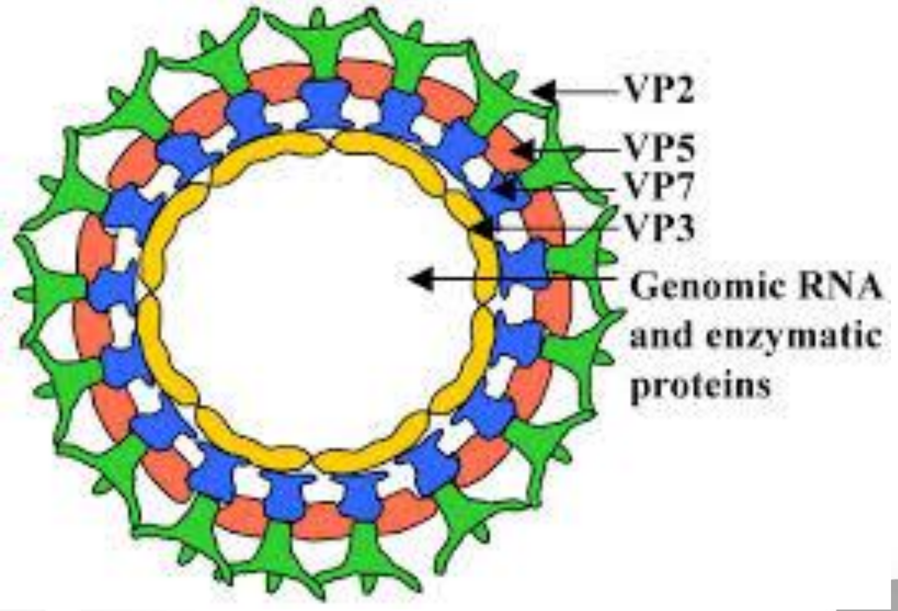
Bluetongue virus: uno, nessuno e centomila

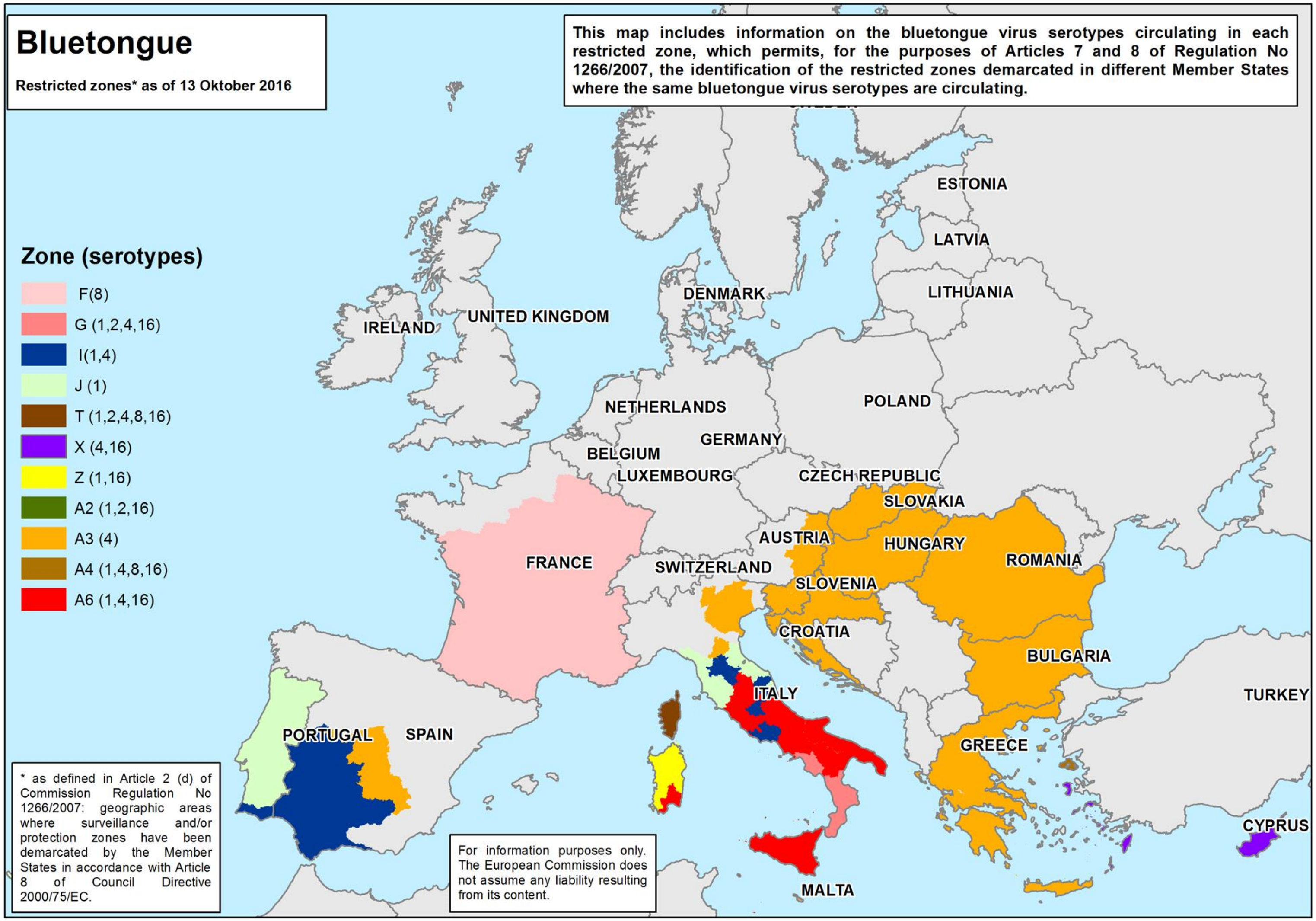
Teramo, 24/11/2016

COVEPI - CESME

Bluetongue

Bluetongue (BT) is a non-contagious (?), viral disease affecting domestic and wild ruminants
(primarily sheep and including cattle, goats, buffalo, antelope, deer, elk and camels) that is transmitted by insects, particularly biting midges of *Culicoides*





Bluetongue

Restricted zones* as of 13 Oktober 2016

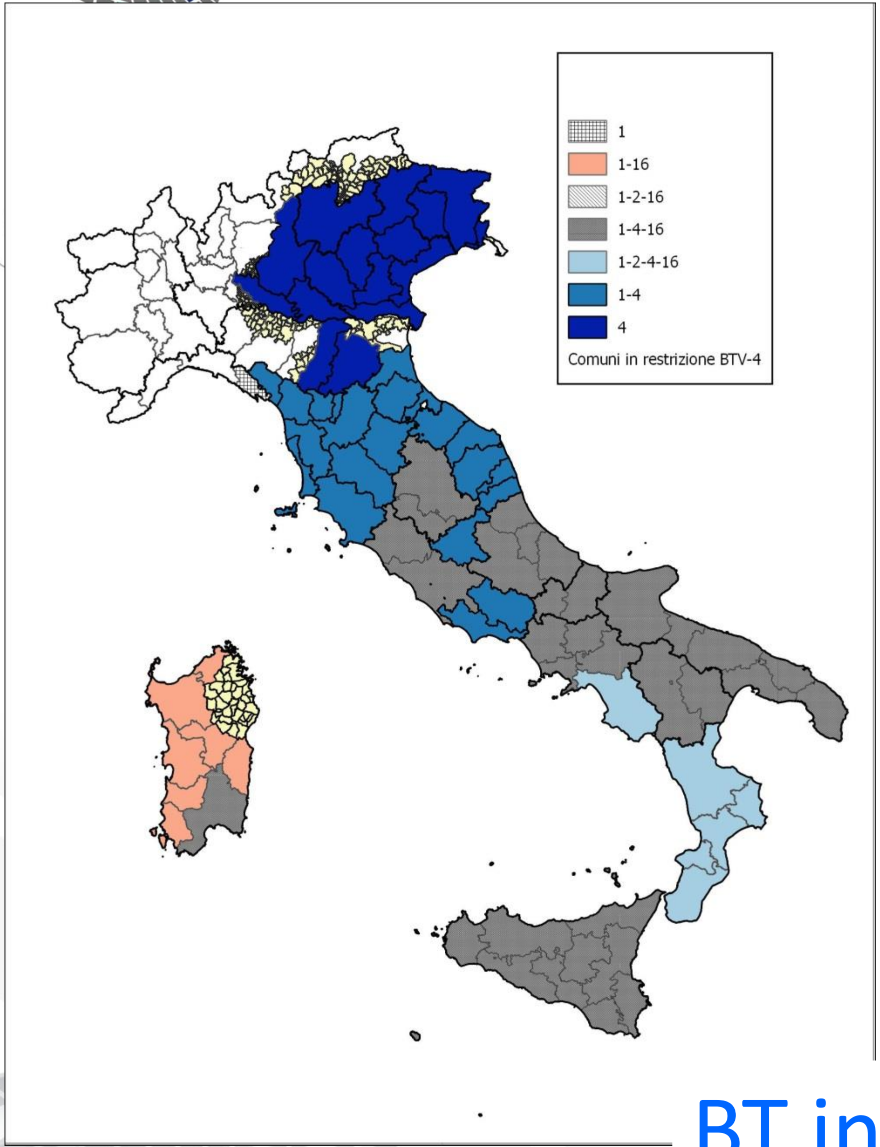
This map includes information on the bluetongue virus serotypes circulating in each restricted zone, which permits, for the purposes of Articles 7 and 8 of Regulation No 1266/2007, the identification of the restricted zones demarcated in different Member States where the same bluetongue virus serotypes are circulating.

* as defined in Article 2 (d) of Commission Regulation No 1266/2007: geographic areas where surveillance and/or protection zones have been demarcated by the Member States in accordance with Article 8 of Council Directive 2000/75/EC.

For information purposes only. The European Commission does not assume any liability resulting from its content.

BT in Europe

BT in Italy


▦ 1
🟧 1-16
▨ 1-2-16
■ 1-4-16
■ 1-2-4-16
■ 1-4
■ 4
Comuni in restrizione BTV-4

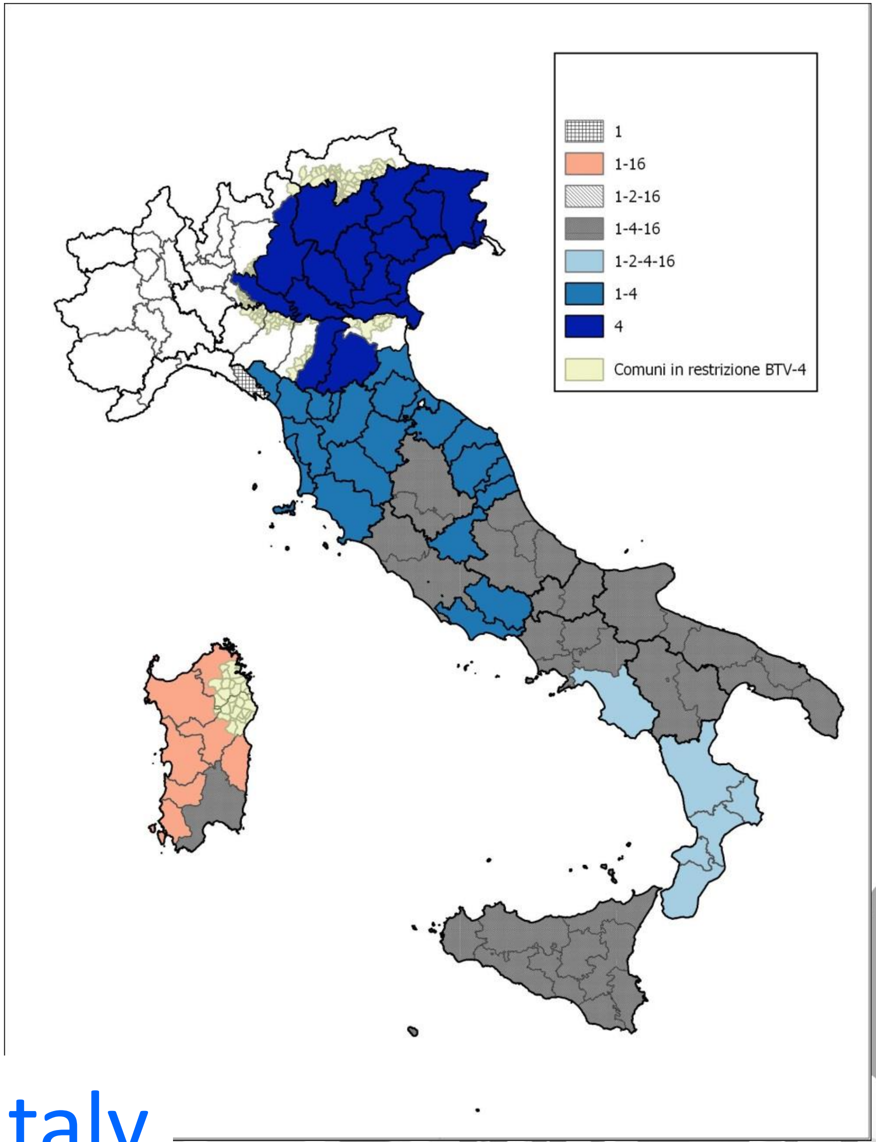

▦ 1
🟧 1-16
▨ 1-2-16
■ 1-4-16
■ 1-2-4-16
■ 1-4
■ 4
□ Comuni in restrizione BTV-4

Fig. 1: Distribuzione geografica degli focolai d BTV8 in Francia (Fonte: OIE)

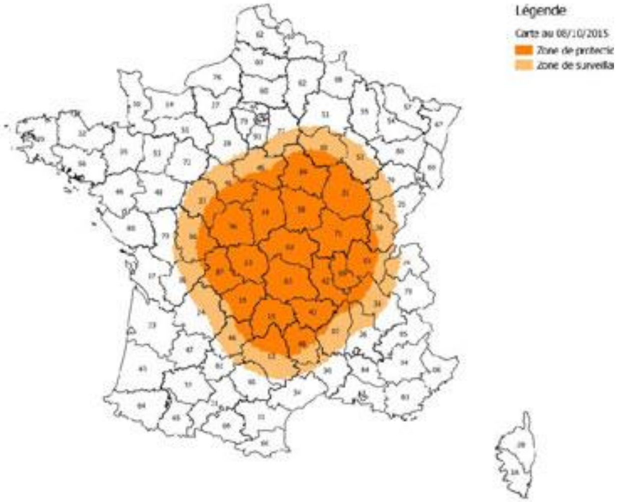
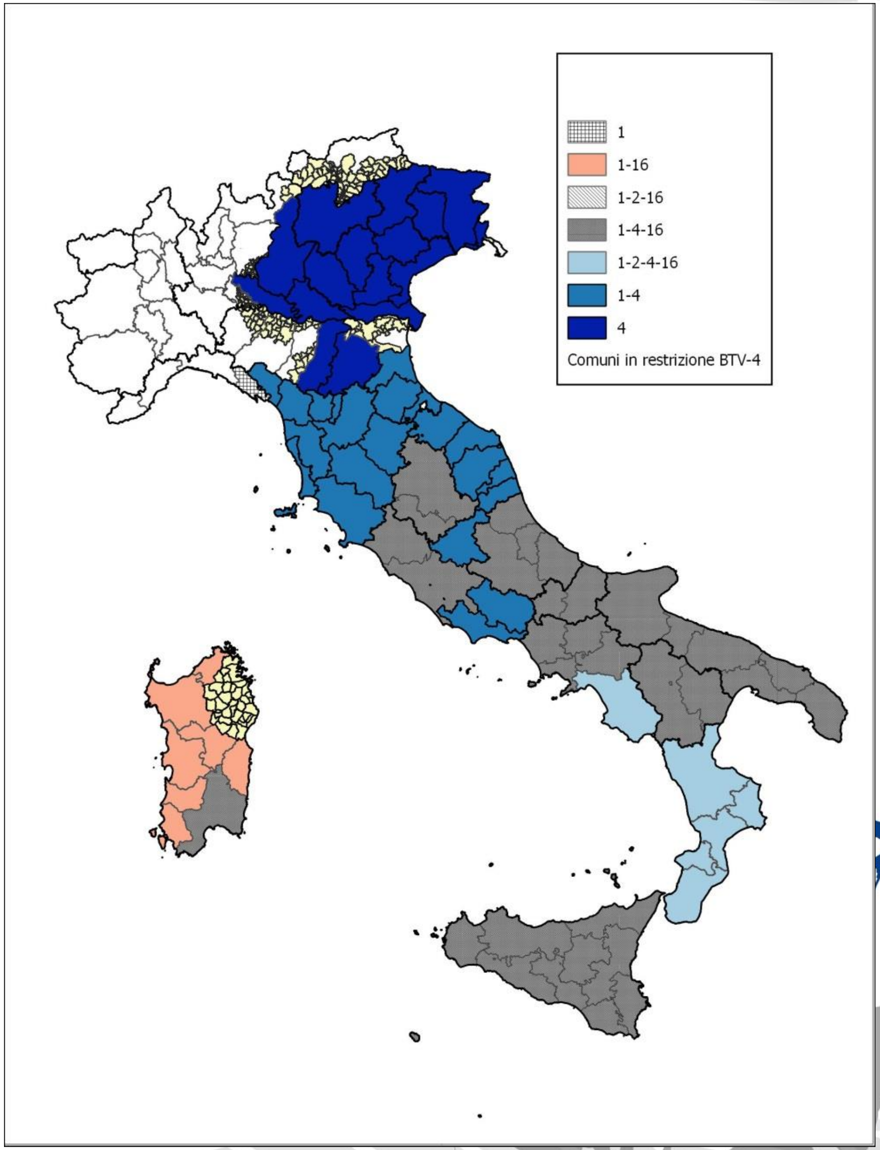
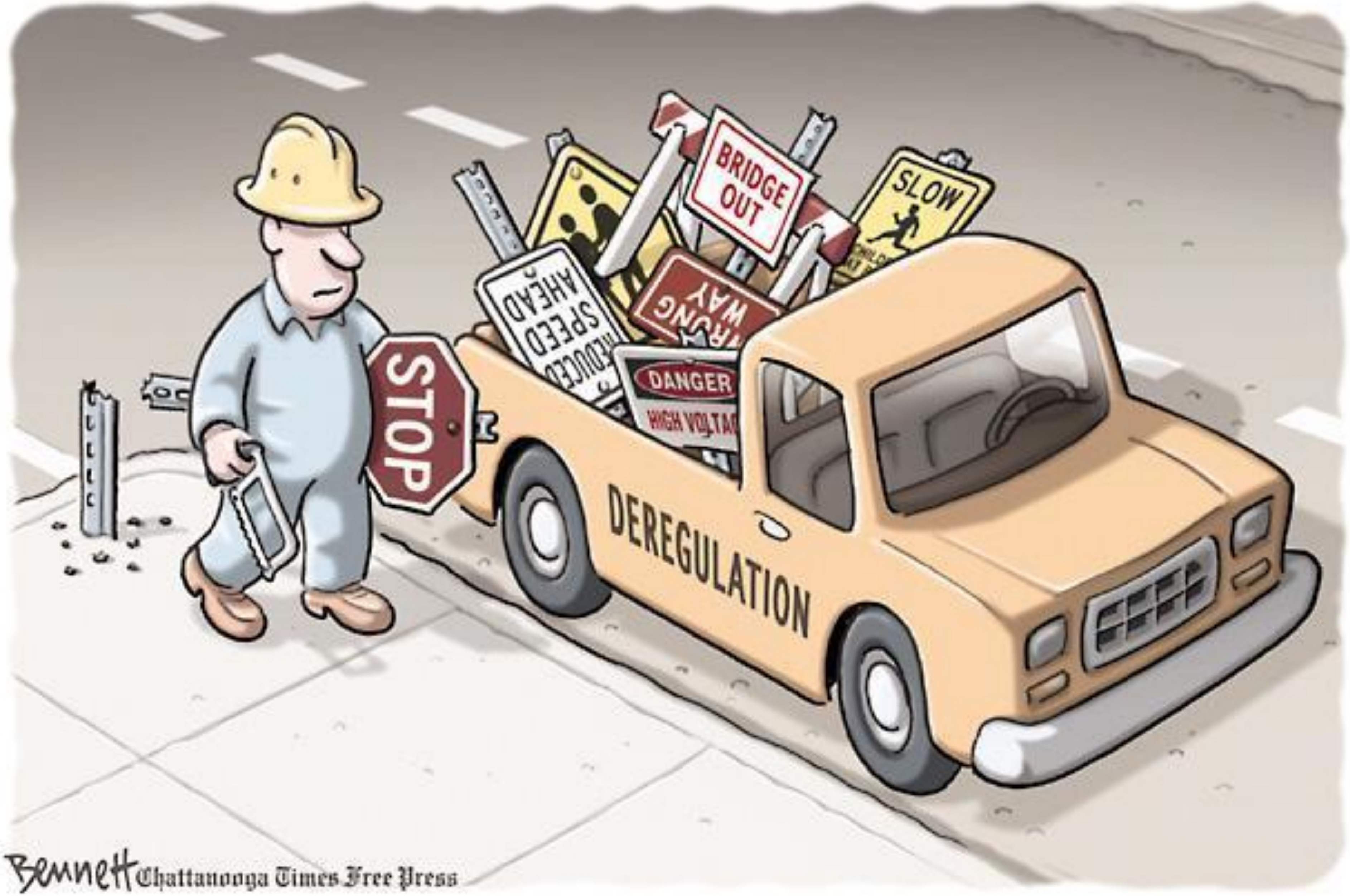
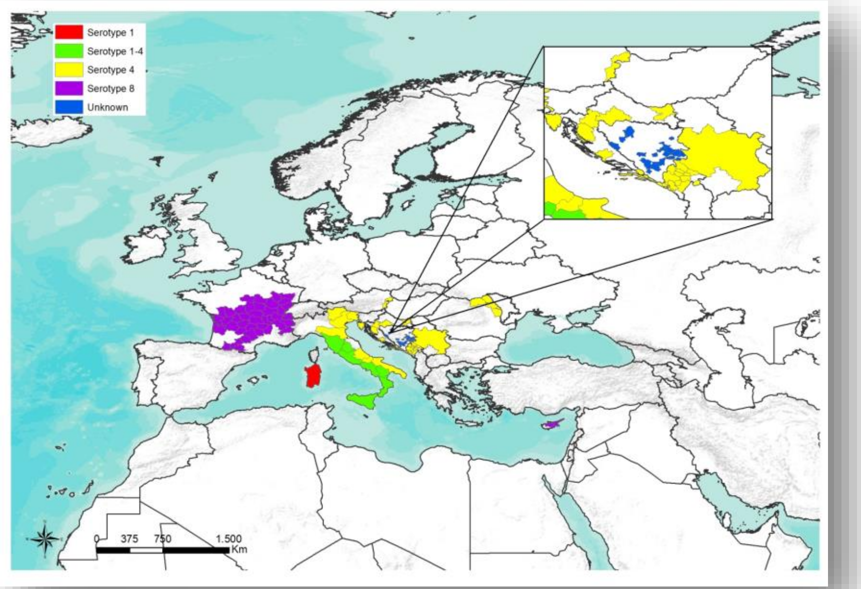


Fig. 2: Delimitazione delle zone di protezione e delle zone di sorveglianza in Francia (Fonte: OIE)





© Clay Bennett.



BT- Epidemiological situation in Europe - 2016

01/01/2016 – 15/11/2016

Oggi la commissione europea sta seriamente pensando di **derubricare** la bluetongue

Fonte dati: OIE e SIMAN

Fig. 1: Distribuzione geografica degli focolai d BTV8 in Francia (Fonte: OIE)

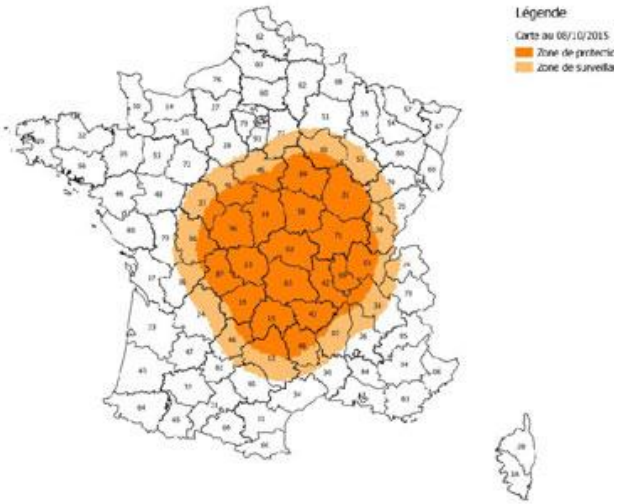


Fig. 2: Delimitazione delle zone di protezione e delle zone di sorveglianza in Francia (Fonte: OIE)

The virus is the same of the 2006 outbreak

Breard et al., 2016

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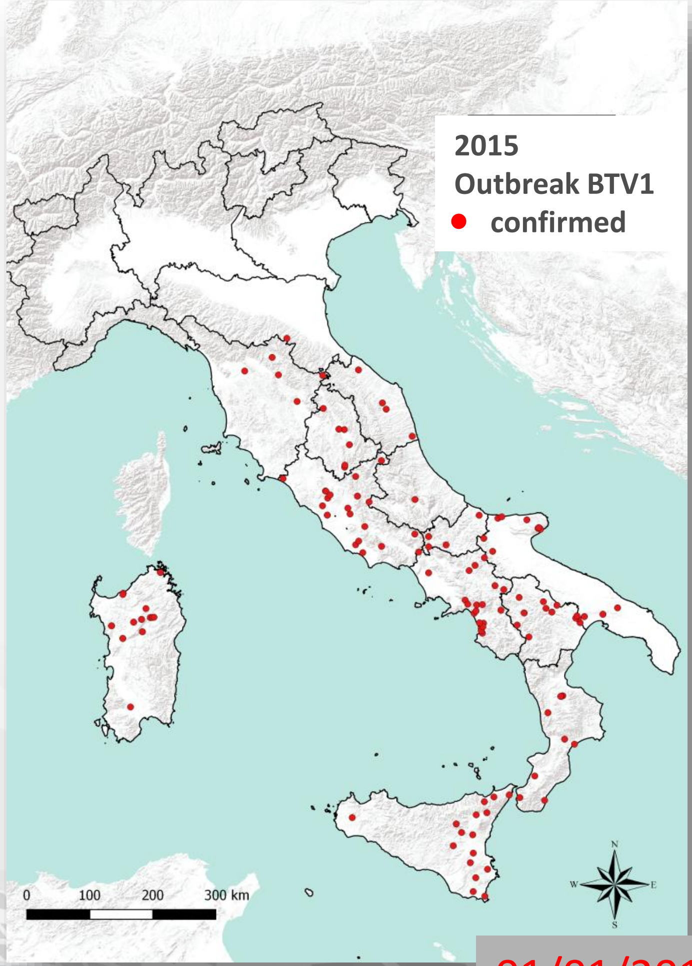
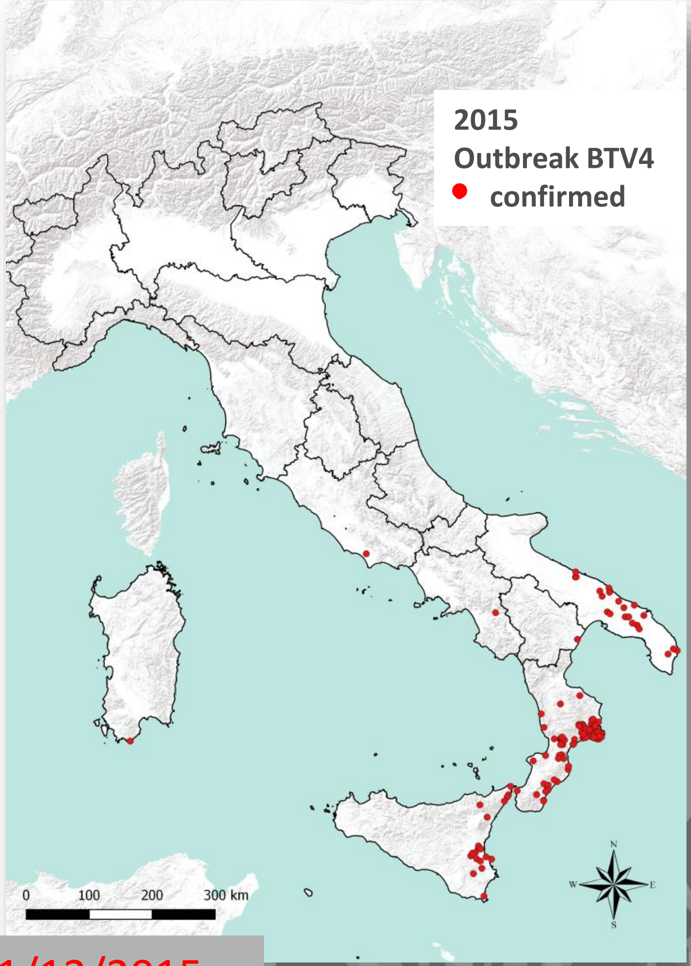
BT- Epidemiological Situation in Italy in 2015

Source: SIMAN

BTV 1



BTV 4

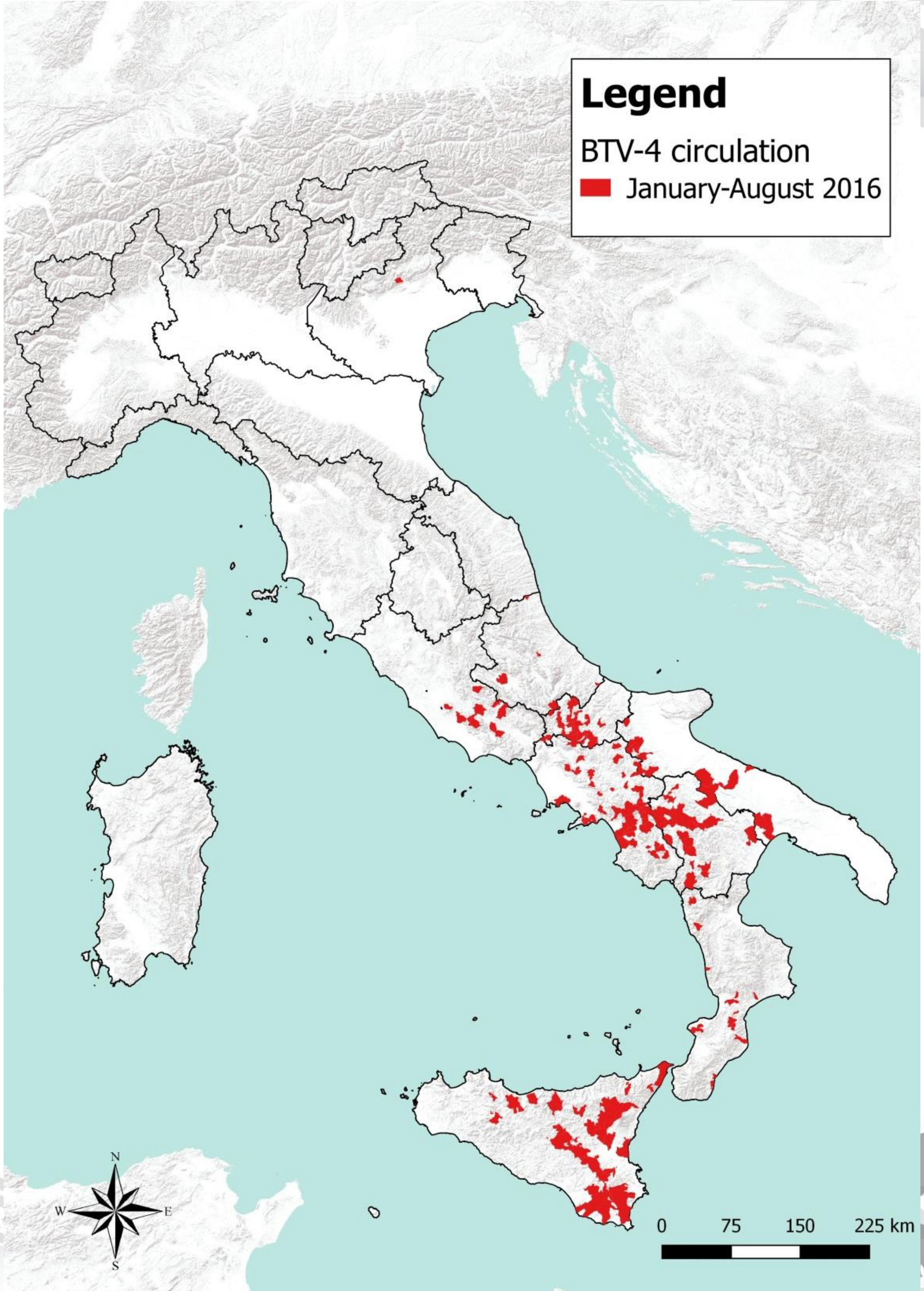


01/01/2015 – 31/12/2015

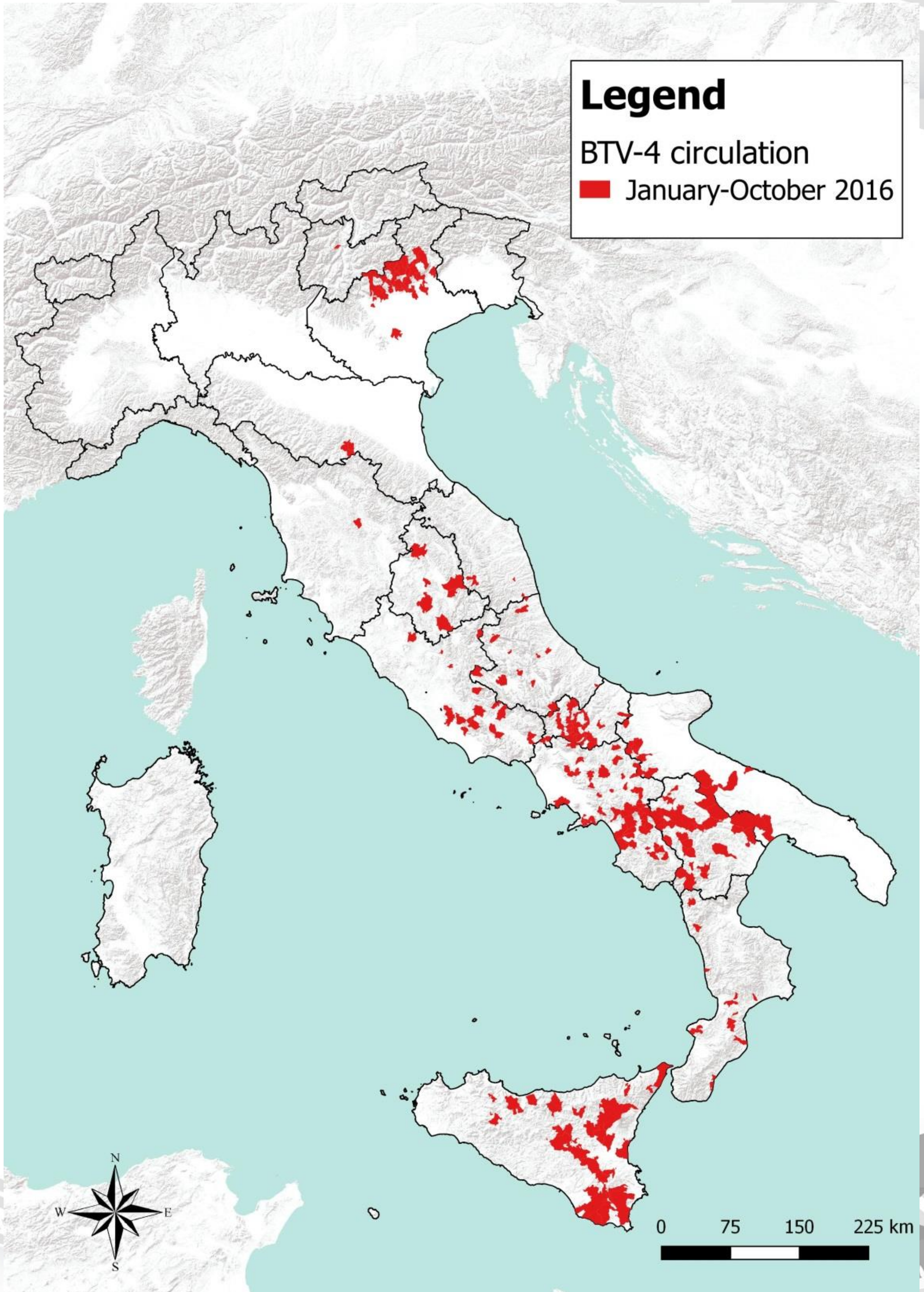
BTV- 4 spreading in Italy in 2016

BTV- 4 spreading in Italy in 2016

BTV- 4 spreading in Italy in 2016



BTV- 4 spreading in Italy in 2016



BT- Epidemiological situation in Italy clinical outbreaks

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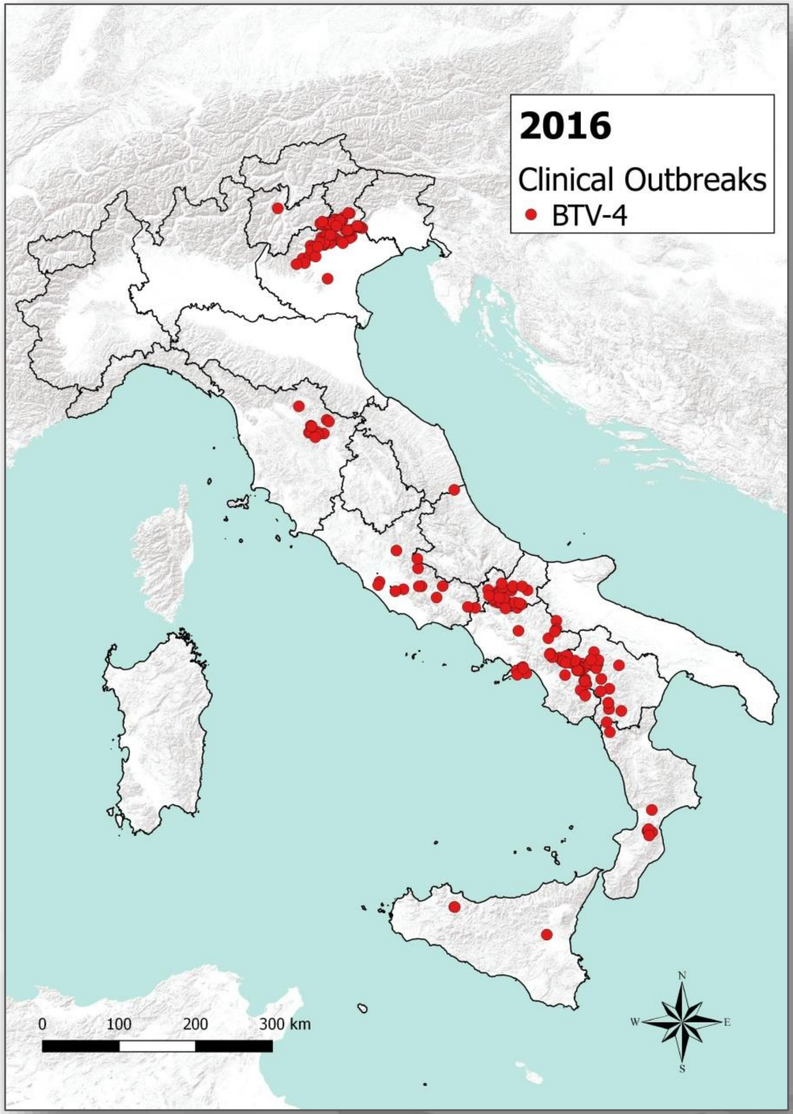
Source:
SIMAN



2016

01/01/2016 – 15/11/2016

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BT- Epidemiological situation in Italy Confirmed and suspected outbreaks– 1/01/2016-29/09/2016

2016

Region	Sierotype	Outbreaks		
		Confirmed	Suspected	Total
ABRUZZO	BTV 1		3	3
	BTV 4	14	5	19
BASILICATA	Serotyping is ongoing	3		3
	BTV 1	11	4	15
	BTV 4	86	8	94
CALABRIA	BTV 1	4	2	6
	BTV 4	38	5	43
CAMPANIA	Serotyping is ongoing	7	18	25
	BTV 1	34	2	36
	BTV 4	94	13	107
EMILIA ROMAGNA	Serotyping is ongoing		1	1
	BTV 4	16		16
FRIULI VENEZIA GIULIA	Serotyping is ongoing		2	2
	BTV 4	1		1
LAZIO	BTV 1	8		8
	BTV 4	27	5	32
LOMBARDIA	Serotyping is ongoing		2	2
MARCHE	Serotyping is ongoing		1	1
	BTV 1		1	1
	BTV 4	5	2	7
MOLISE	Serotyping is ongoing		2	2
	BTV 1		1	1
	BTV 4	53	7	60
	BTV 9		1	1
PUGLIA	Serotyping is ongoing	1	2	3
	BTV 4	24	5	29
SARDEGNA	Serotyping is ongoing		4	4
	BTV 1	7	2	9
SICILIA	Serotyping is ongoing	1	4	5
	BTV 1	10	3	13
	BTV 4	76	6	82
TOSCANA	Serotyping is ongoing		8	8
	BTV 1	2		2
	BTV 4	23		23
TRENTO	BTV 4	6		6
UMBRIA	BTV 1	1		1
	BTV 4	7		7
VENETO	BTV 4	318		318
Total		877	119	996

Source: SIMAN

COVEPI - CESME

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Source: SIMAN

2016

BT- Epidemiological situation in Italy confirmed outbreaks– 1/01/2016-15/11/2016

Region	Serotype	Confirmed outbreaks	CATTLE/BUFFALOES				SHEEP/GOATS				Muflon			
			Susceptible	Cases	Clinically affected	Deaths	Susceptible	Cases	Clinically affected	Deaths	Susceptible	Cases	Clinically affected	Deaths
ABRUZZO	BTV 4	14	375	12	0	0	1091	31	17	16				
BASILICATA	Serotyping is ongoing	3	171	3	0	0	231	65	64	10				
	BTV 1	11	2173	16	0	0	260	3	0	0				
	BTV 4	86	3045	58	0	0	5609	290	183	120				
CALABRIA	BTV 1	4	83	3	0	0	60	1	0	0				
	BTV 4	38	362	27	0	0	2263	154	67	38				
CAMPANIA	Serotyping is ongoing	7					1569	82	82	14				
	BTV 1	34	1145	28	0	0	43	43	0	0				
	BTV 4	94	2001	160	41	0	8651	556	508	152				
EMILIA ROMAGNA	BTV 4	16	1161	55	0	0								
FRIULI VENEZIA GIULIA	BTV 4	1					47	6	5	1				
LAZIO	BTV 1	8	1293	10	1	0	243	2	0	0				
	BTV 4	27	1196	22	1	0	5531	53	47	18				
MARCHE	BTV 4	5	51	1	0	0	397	13	10	2				
MOLISE	BTV 4	53	629	28	0	0	15830	814	799	463				
PUGLIA	Serotyping is ongoing	1					37	1	0	0				
	BTV 4	24	876	64	0	0	42	2	1	0				
SARDINIA	BTV 1	7	147	3	1	0	2104	8	0	0				
SICILY	Serotyping is ongoing	1	21	1	0	0								
	BTV 1	10	589	23	0	0								
	BTV 4	76	3945	281	7	0	1966	76	70	1				
TUSCANY	BTV 1	2	33	1	0	0	3	0	0	0				
	BTV 4	23	215	14	13	12	2343	114	70	37				
TRENTO	BTV 4	6	138	12	0	0	42	2	2	1	1	1	1	1
UMBRIA	BTV 1	1	91	1	0	0								
	BTV 4	7	533	10	0	0								
VENETO	BTV 4	318	17042	1539	8	1	5074	125	111	21	2	2	2	2
Total		877	37315	2372	72	13	53436	2441	2036	894	3	3	3	3

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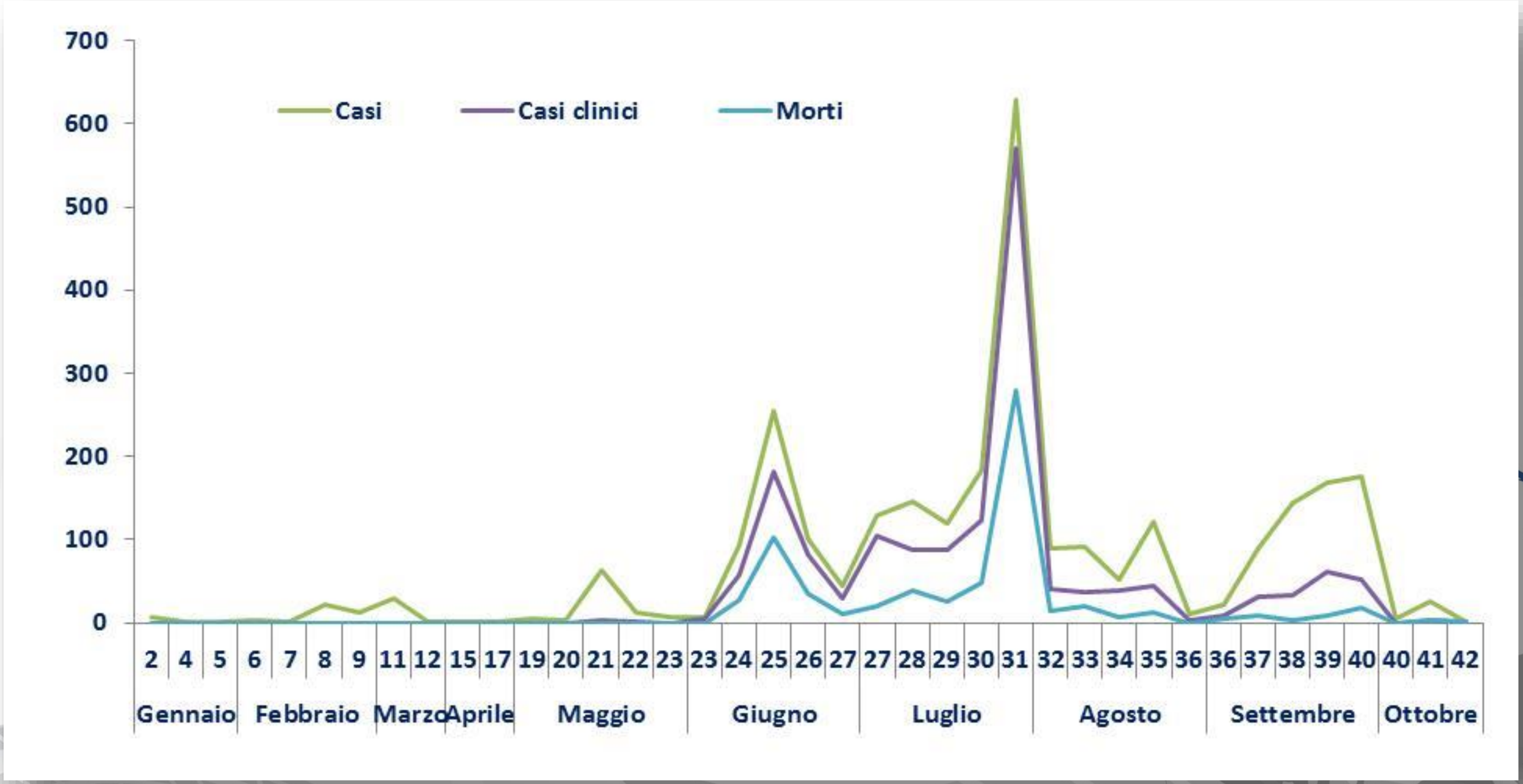
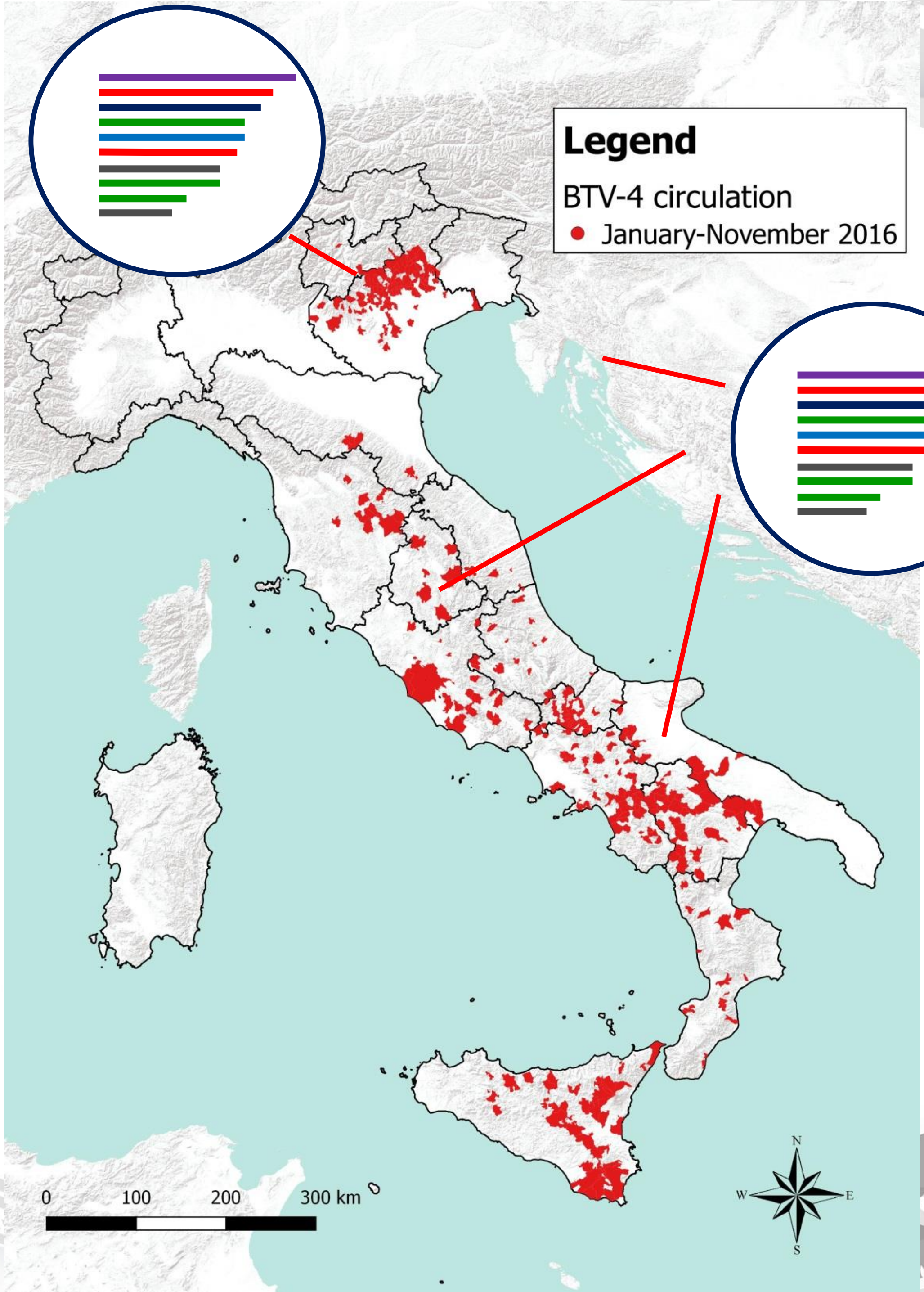
BT- andamento temporale in Italia BTV 4
1/01/2016 - 18/10/2016

BTV4



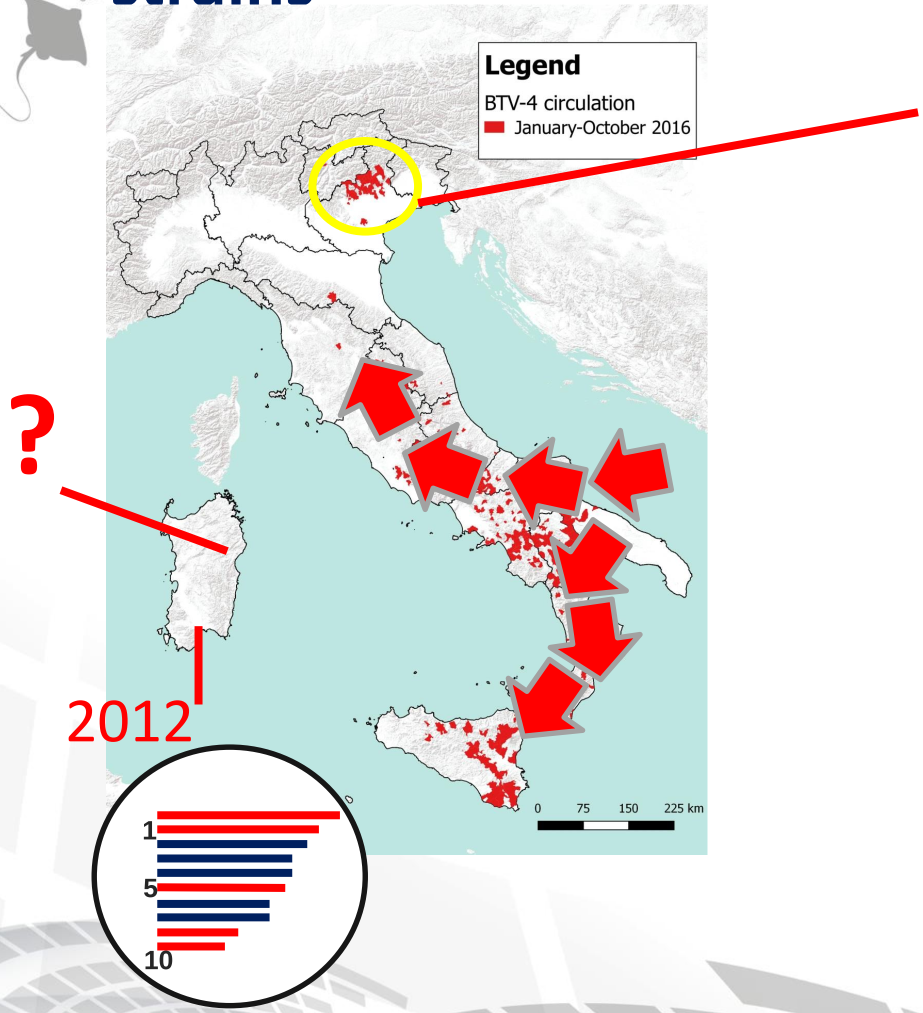
BT- 4 spreading in Italy in 2016

BTV 1 and BTV 4



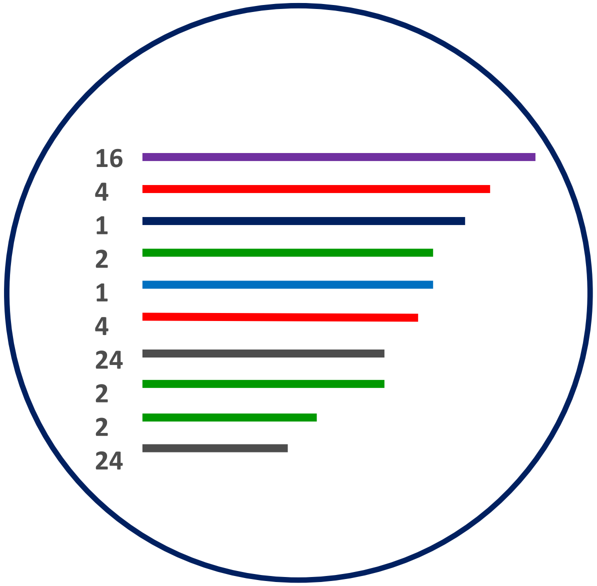
01/01/2016
-
15/11/2016

Origin of Italian BTV-4 2016 strains



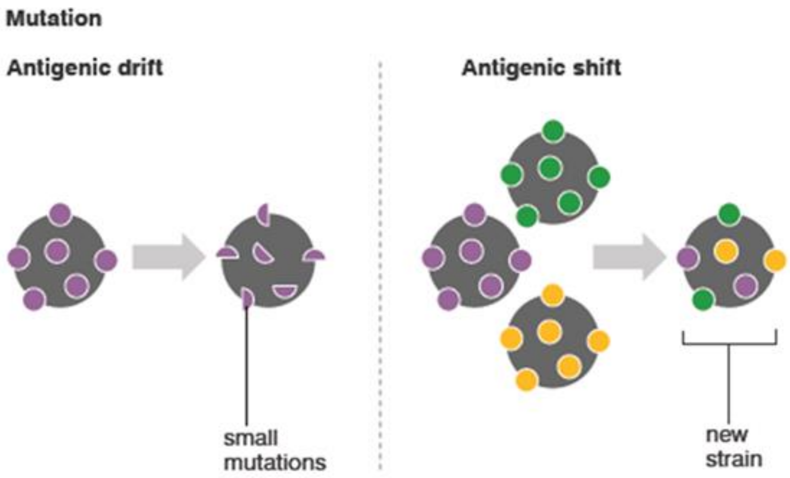
- Assess the real origin of BTV-4 strains is impossible
- Low intra variant divergence usually observed in western strains
- Lack of sequences from Balkans
- Deeper parental strain analysis is ongoing
- Sequencing of additional strains is in process
- Genomes of Croatia, Puglia, Molise, Marche, Emilia and Veneto strains have been fullly sequenced

All western topotype segments



BTV-4 Balkans

BTV-1, BTV-2, BTV-4, BTV-16 and BTV-24

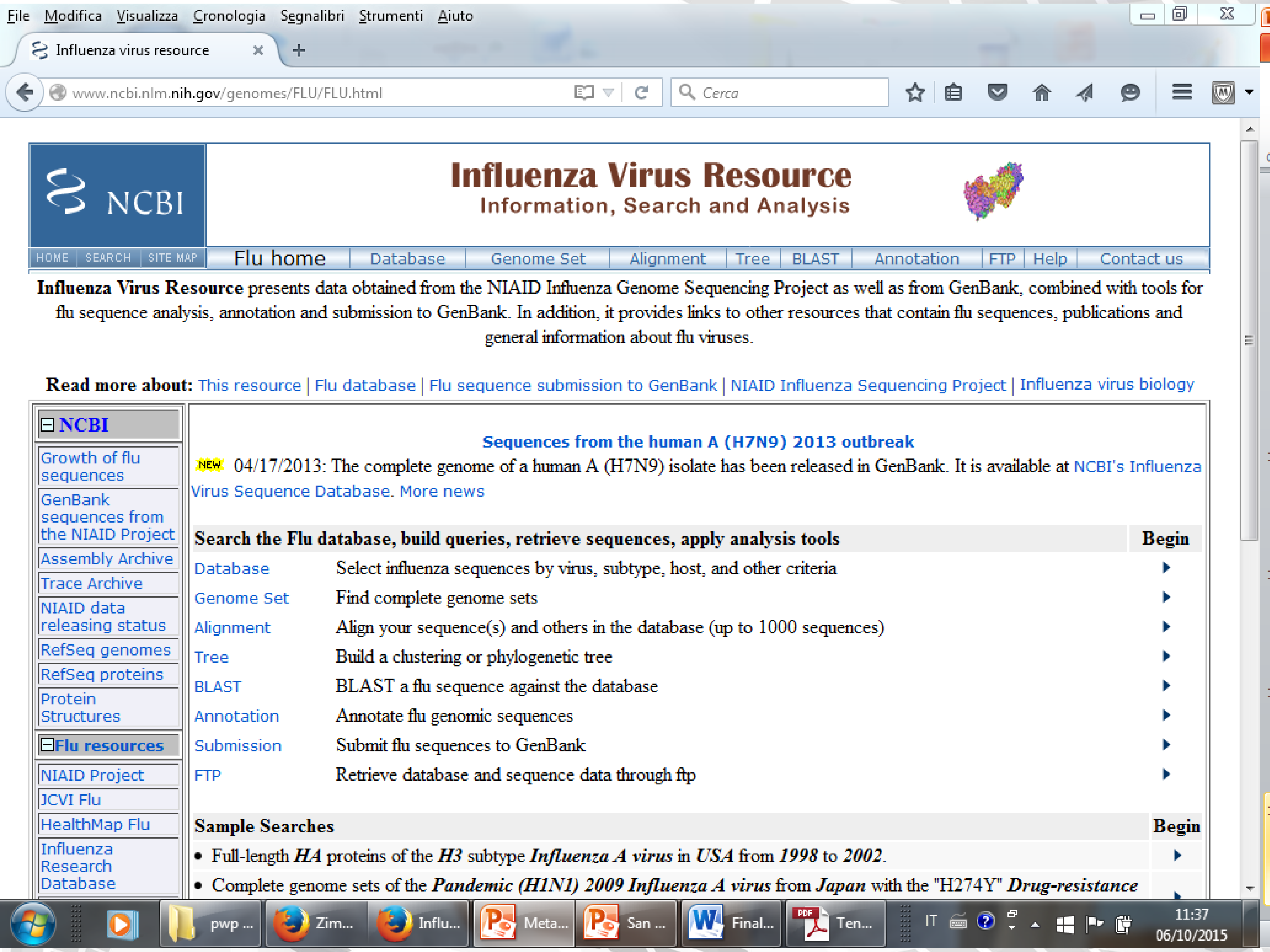


- **Several natural western/eastern topotype strains exist**

- **Reassortment is easier if strains belong to the same topotype (*Shaw et al., 2013*)**

- **BTV-1 and BTV-26 can reassort (*in vitro*; *Pullinger et al., 2016*)**

- **BTV-26 in Mauritania (*Lorusso et al., 2016*)**



Influenza Virus Resource

Information, Search and Analysis

Flu home | Database | Genome Set | Alignment | Tree | BLAST | Annotation | FTP | Help | Contact us

Influenza Virus Resource presents data obtained from the NIAID Influenza Genome Sequencing Project as well as from GenBank, combined with tools for flu sequence analysis, annotation and submission to GenBank. In addition, it provides links to other resources that contain flu sequences, publications and general information about flu viruses.

Read more about: This resource | Flu database | Flu sequence submission to GenBank | NIAID Influenza Sequencing Project | Influenza virus biology

⊟ **NCBI**

- Growth of flu sequences
- GenBank sequences from the NIAID Project
- Assembly Archive
- Trace Archive
- NIAID data releasing status
- RefSeq genomes
- RefSeq proteins
- Protein Structures

⊟ **Flu resources**

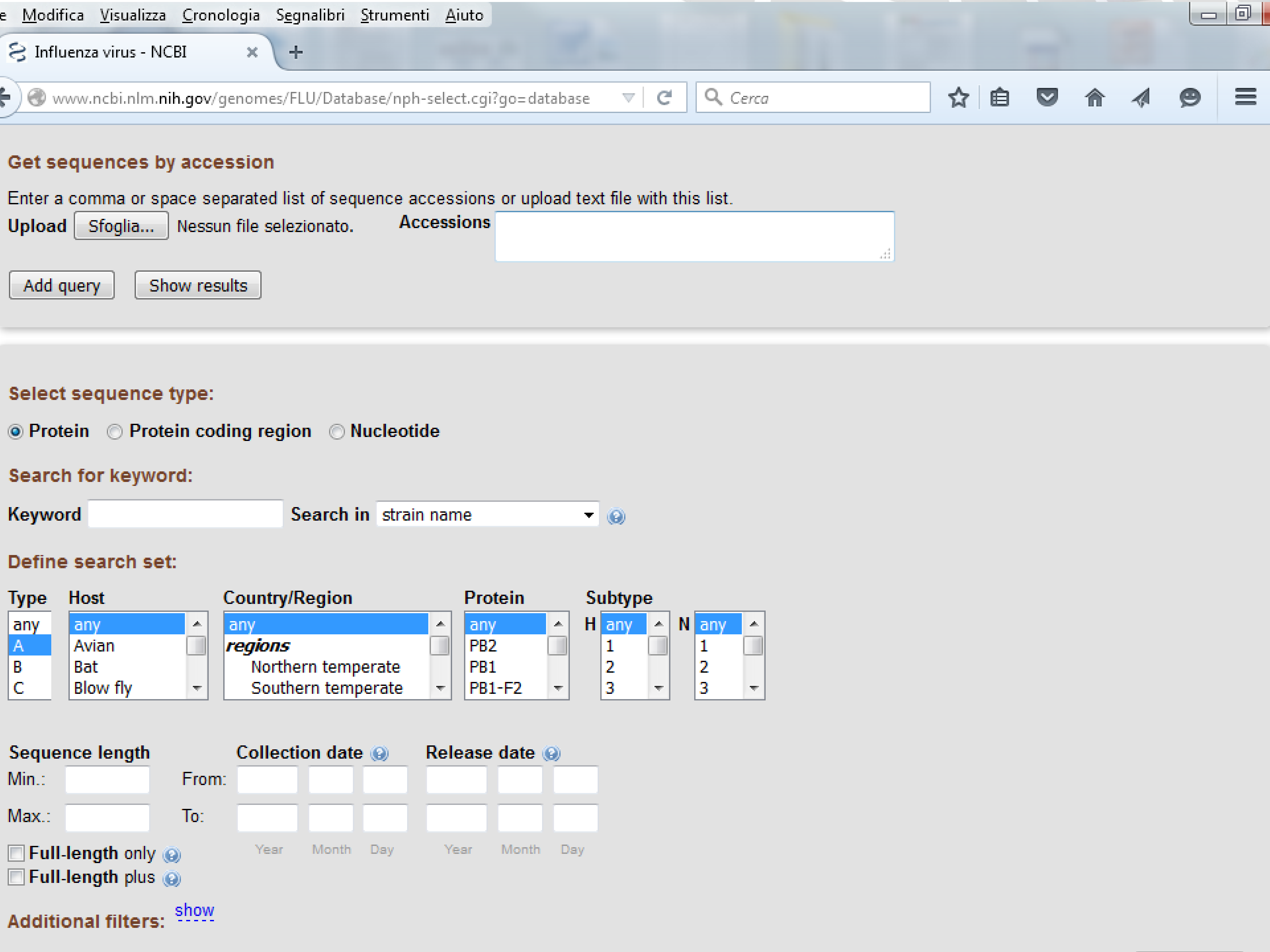
- NIAID Project
- JCVI Flu
- HealthMap Flu
- Influenza Research Database

Sequences from the human A (H7N9) 2013 outbreak

NEW 04/17/2013: The complete genome of a human A (H7N9) isolate has been released in GenBank. It is available at NCBI's Influenza Virus Sequence Database. More news

Search the Flu database, build queries, retrieve sequences, apply analysis tools		Begin
Database	Select influenza sequences by virus, subtype, host, and other criteria	▸
Genome Set	Find complete genome sets	▸
Alignment	Align your sequence(s) and others in the database (up to 1000 sequences)	▸
Tree	Build a clustering or phylogenetic tree	▸
BLAST	BLAST a flu sequence against the database	▸
Annotation	Annotate flu genomic sequences	▸
Submission	Submit flu sequences to GenBank	▸
FTP	Retrieve database and sequence data through ftp	▸

Sample Searches	Begin
• Full-length *HA* proteins of the *H3* subtype *Influenza A virus* in *USA* from *1998* to *2002*.	▸
• Complete genome sets of the *Pandemic (H1N1) 2009 Influenza A virus* from *Japan* with the "H274Y" *Drug-resistance*	▸



www.ncbi.nlm.nih.gov/genomes/FLU/Database/nph-select.cgi?go=database

Get sequences by accession

Enter a comma or space separated list of sequence accessions or upload text file with this list.

Upload Sfoglia... Nessun file selezionato. **Accessions**

Select sequence type:

● **Protein** ○ **Protein coding region** ○ **Nucleotide**

Search for keyword:

Keyword **Search in** strain name

Define search set:

Type: any, A, B, C

Host: any, Avian, Bat, Blow fly

Country/Region: any, *regions*, Northern temperate, Southern temperate

Protein: any, PB2, PB1, PB1-F2

Subtype

H: any, 1, 2, 3

N: any, 1, 2, 3

Sequence length

Min.:

Max.:

Collection date

From: Year Month Day

To: Year Month Day

Release date

From: Year Month Day

To: Year Month Day

☐ **Full-length** only

☐ **Full-length** plus

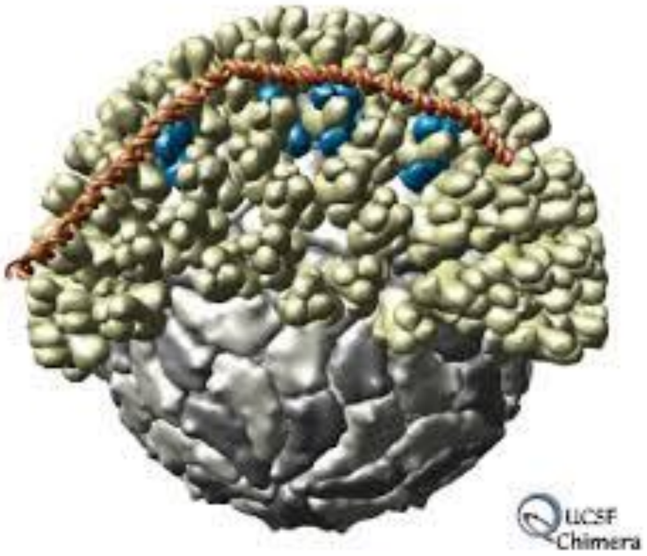
Additional filters: show

New BTV serotypes

- Five new serotypes (BTV 25, 26, 27, 28 and 29) have been identified since 2008, 3 of them in the last two years only. Probably more….

- The chicken and the egg story ?

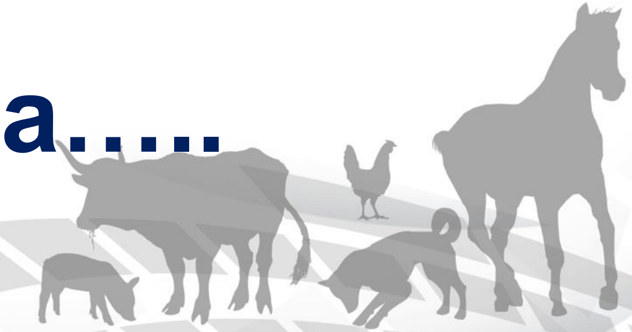
BREAKING NEWS



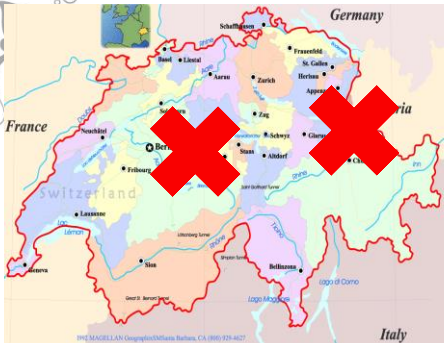
BTV-28

BTV-29 Alpaca, South Africa

More BTVs from Asia…..



BTV-25



It doesn’t grow *in vitro*

- BTV-25 RNA positive over a period of 19-25 months

- Epidemiology?

Vögtlin A et., 2016; Hofmann et al., 2008

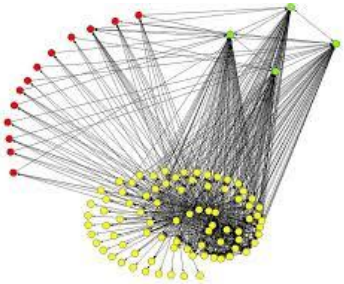
BTV-26

Direct contact

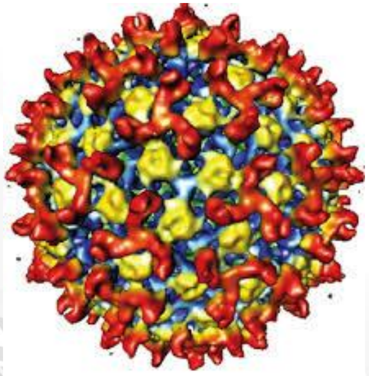
Batten et al., 2013; 2014

BTV-27

Healthy



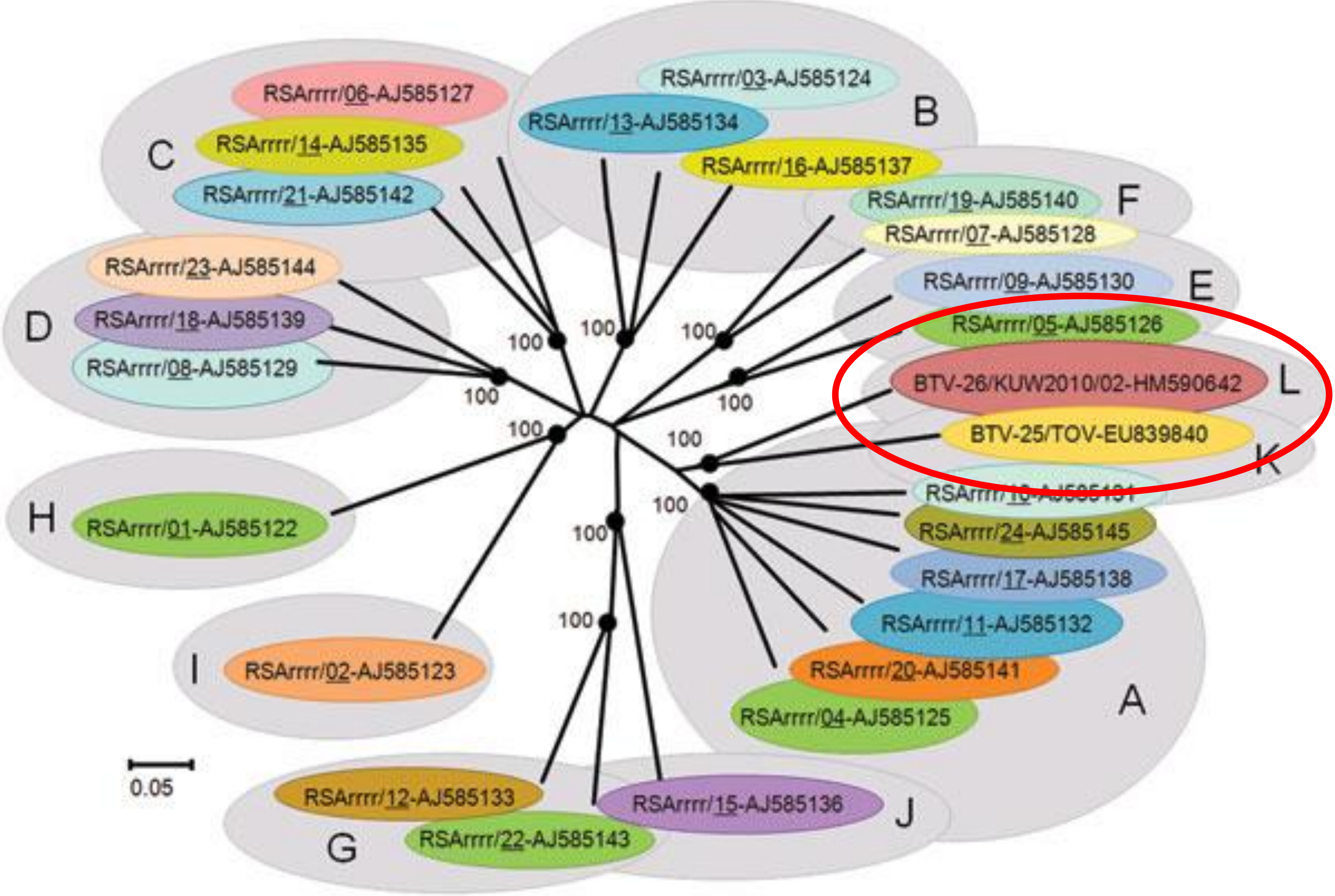
At least 3 variants (92% nt id Seg2)



Single serotype

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

Allineamento sequenze del segmento 2 dei sierotipi del virus della bluetongue.



BTVX ITL2015

CASE STUDY: Sardinia, 2015
BT surveillance plan in Italy

2 Farms:

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!

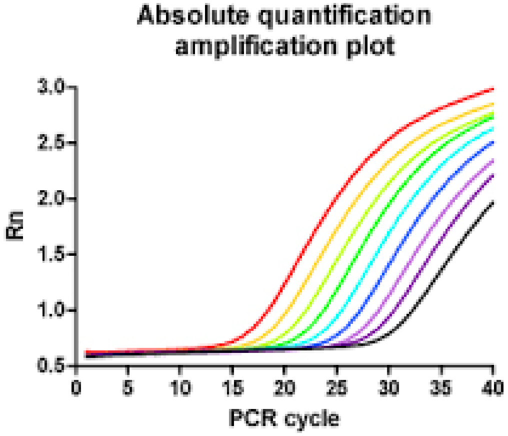
…..from the entire group (17)

Whole blood

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

POS

X 9/17



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

NEG

X 17/17

European strains (Seg2)

NEG

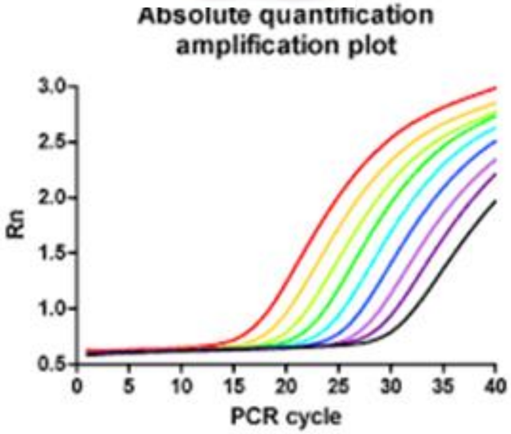
X 17/17

BTVX ITL2015

October 2015

X 8/17 + cElisa, -SN

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

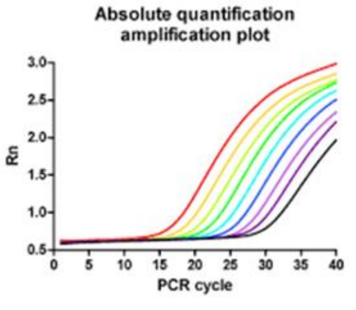


BTVX ITL2015

December 2015

X 90/107 + cElisa, -SN

X 37/107 + RTqPCR $_{1\text{-}27}$; - RTqPCR $_{1\text{-}24}$; -RTqPCR $_{\text{Seg2 ES}}$



Similar scenario in Farm 2

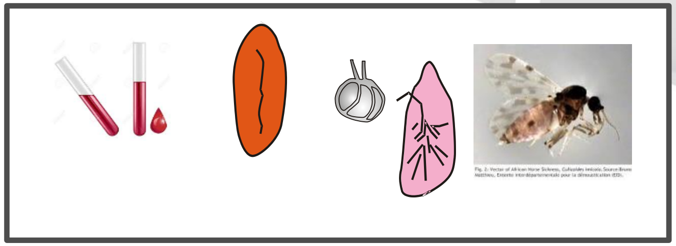
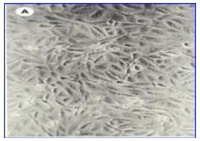


Fig. 2: Vector of African Horse Sickness, *Culicoides imicola*. Source Bruno Mathieu, Entente interdépartementale pour la démoustication (EID).

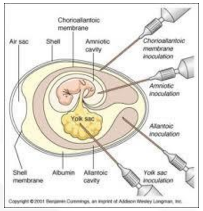
Serogroup identification (1-27)

ISOLATION ← + → **SEROTYPING**



KC, BHK, VERO

or



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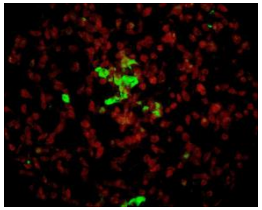
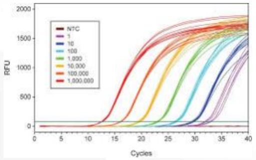
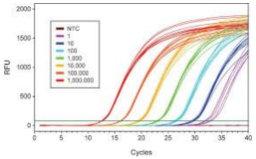


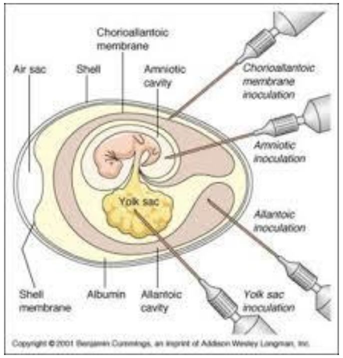


Type specific RT-PCR



BTVX ITL2015

Isolation attempts



ECE

Cell lines
(KC, BHK-21, BSR)

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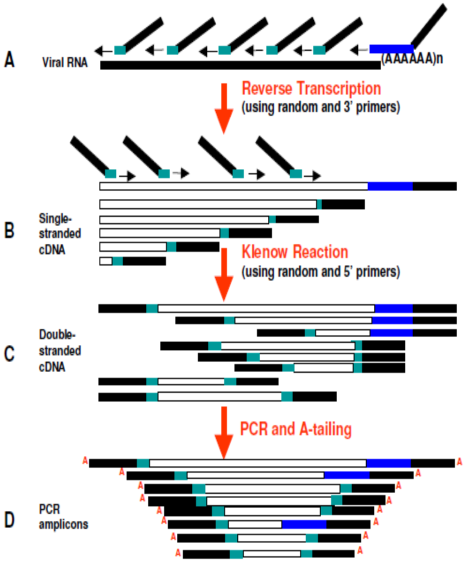
BTVX ITL2015

Centomila

Whole Genome Sequencing by NGS

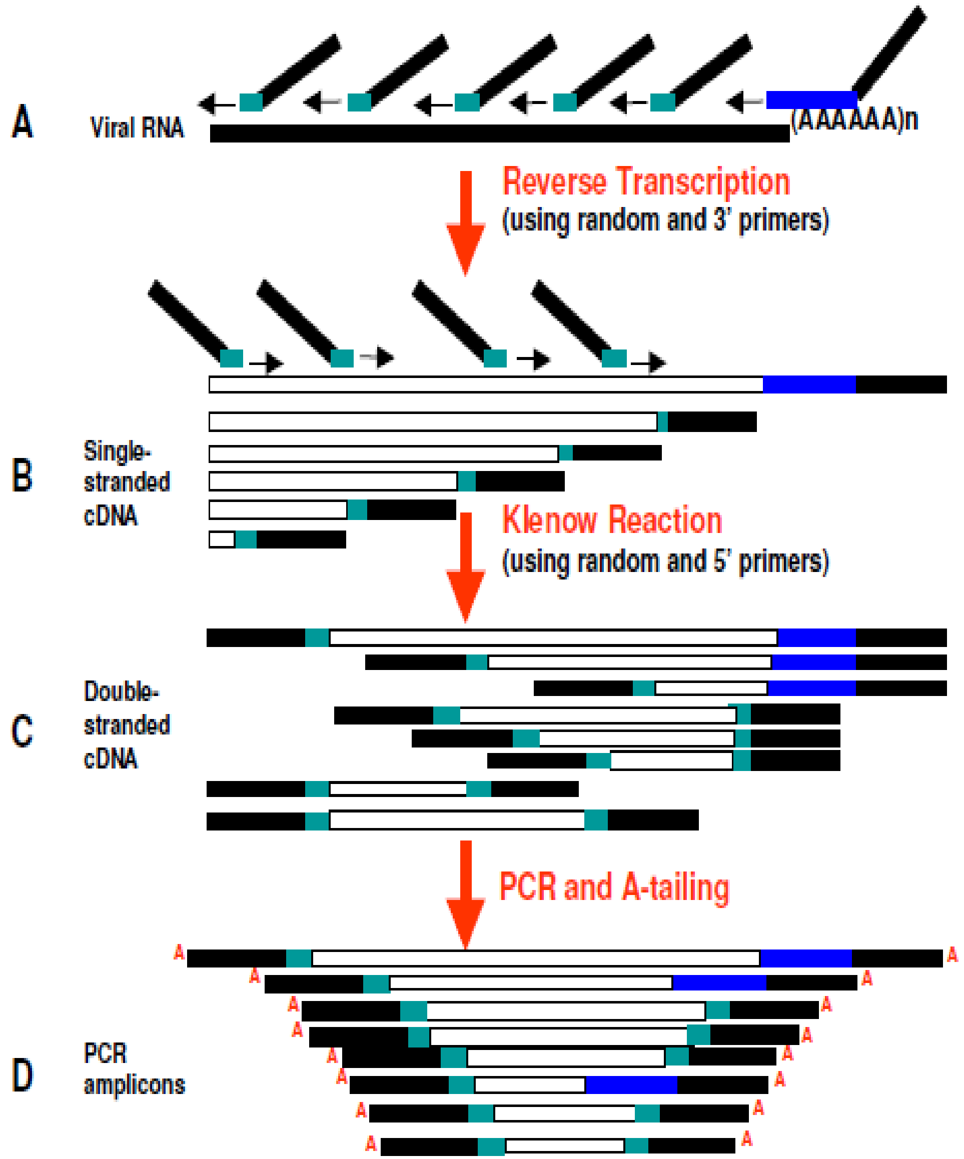
→ **RNA** →



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

SISPA

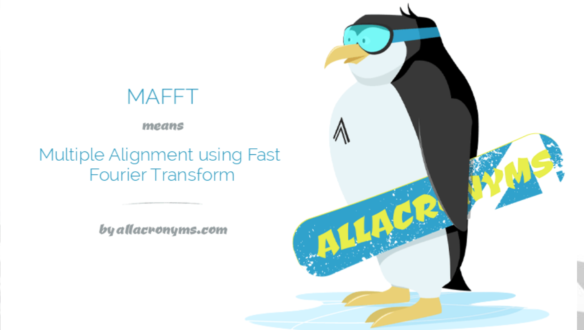
Sequencing Indipendent Single Primer Amplification

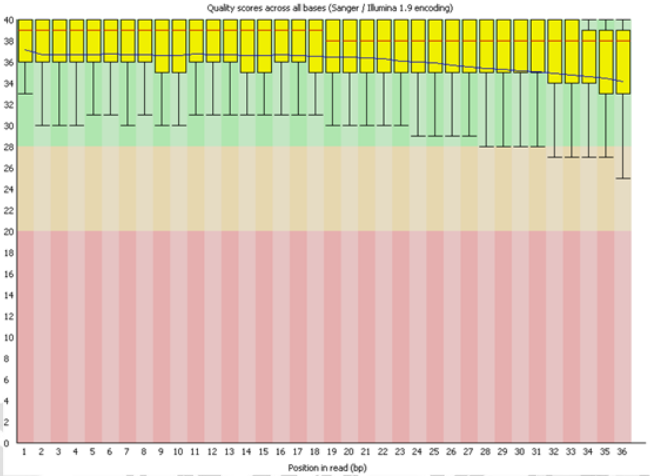


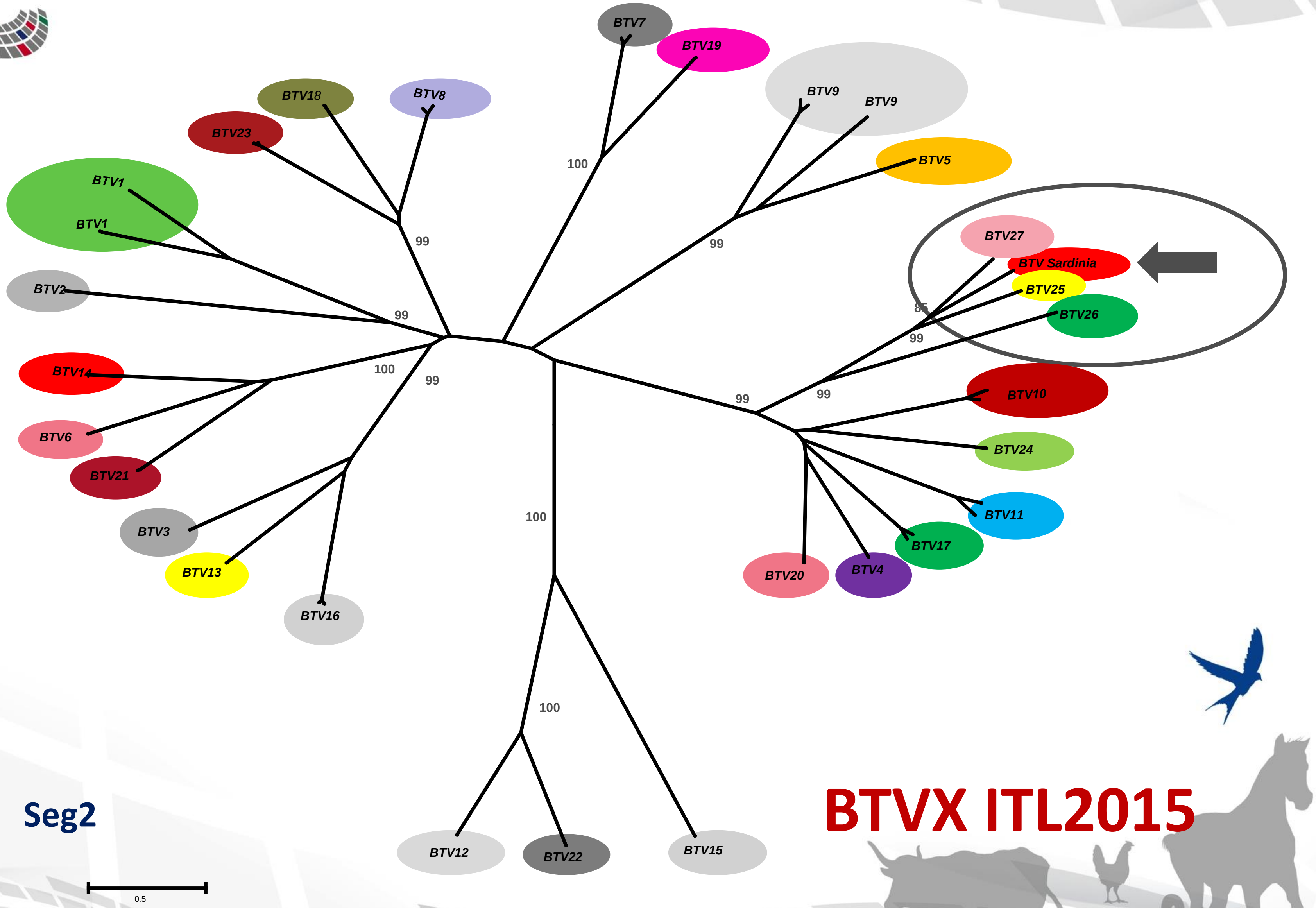
BTVX ITL2015

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

SPAdes







Seg2

BTVX ITL2015

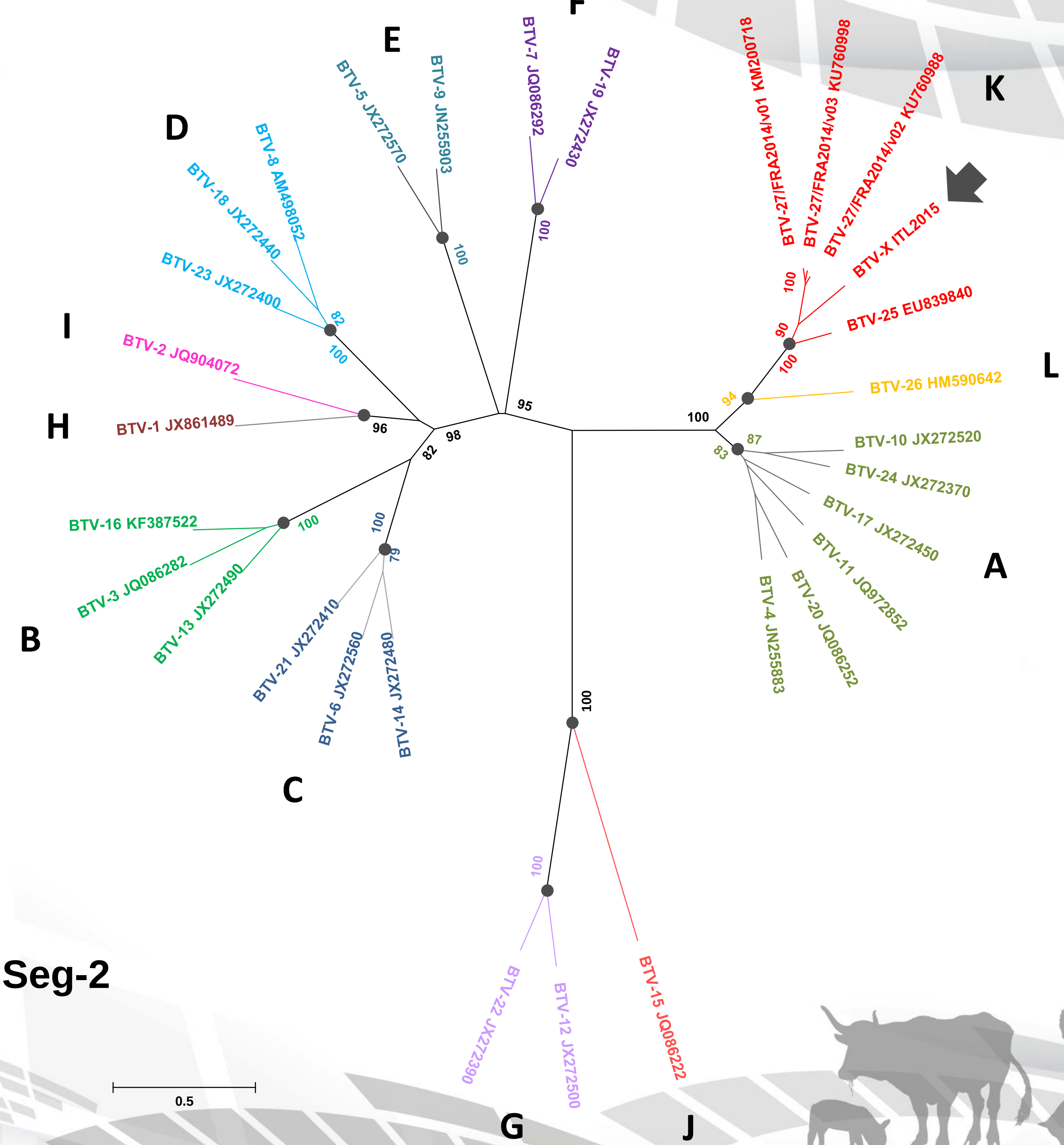
BTV-X ITL2015 Seg 2 VP2 KX234079 -It lacks 16 aa at 5’end and 82 aa at 3’end	BTV-27 KM200718	75.3/77.1
	BTV-27/FRA2014/v02 KU760988	75.3/76.0
	BTV-27/FRA2014/v03	74.2/77.3
	BTV-25 EU839840	73.1/74.0
	BTV-26 KUW2010/02 HM590642	63.1/60.0

Seg 2/ VP 2

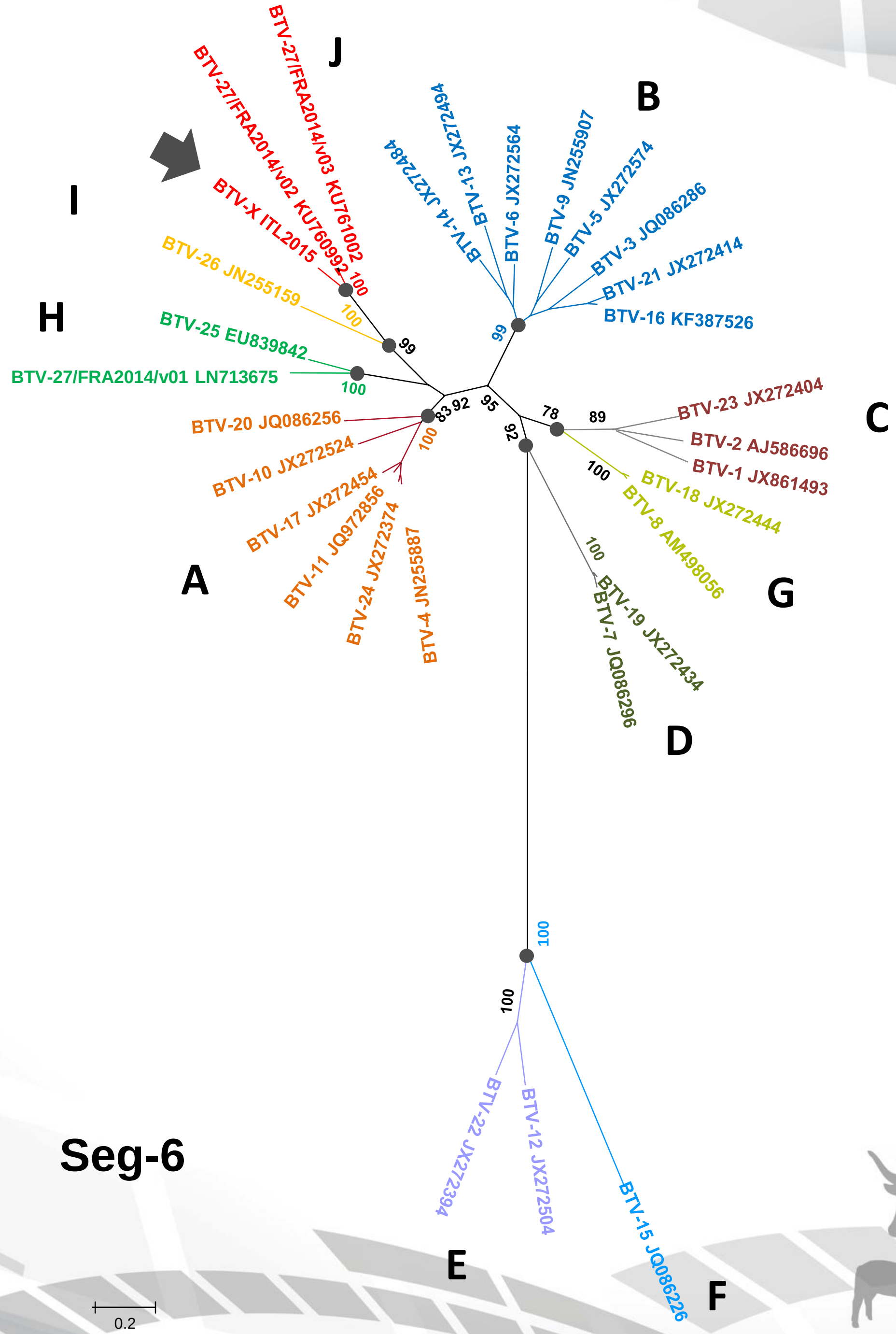
BTV-X ITL2015 Seg 6 VP5 KX234083 -It lacks 39 aa at 5’end -Partial 3’end NCR	BTV-27/FRA2014/v03 KU761002	84.2/93.7
	BTV-27/FRA2014/v02 KU760992	84.2/93.7
	BTV-26 KUW2010/02 JN255159	73.7/81.6
	BTV-25 EU839842	70.2/76.3
	BTV-27 LN713675	70.8/75.3

Seg 6/ VP 5

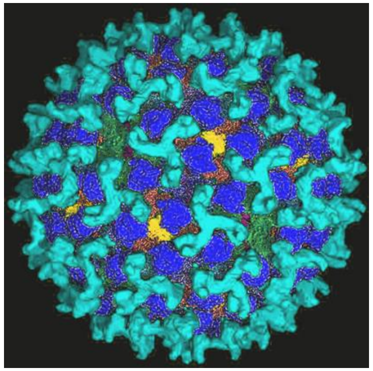
Only Seg 3 and Seg 4 were obtained from farm 2; 100% nt id with Farm 1


Seg-2
A
BTV-10 JX272520
BTV-24 JX272370
BTV-17 JX272450
BTV-11 JQ972852
BTV-20 JQ086252
BTV-4 JN255883
87
83
B
BTV-16 KF387522
BTV-3 JQ086282
BTV-13 JX272490
100
C
BTV-21 JX272410
BTV-6 JX272560
BTV-14 JX272480
100
79
D
BTV-8 AM498052
BTV-18 JX272440
BTV-23 JX272400
82
100
E
BTV-5 JX272570
BTV-9 JN255903
100
F
BTV-7 JQ086292
BTV-19 JX272430
100
G
BTV-22 JX272390
BTV-12 JX272500
100
H
BTV-1 JX861489
96
I
BTV-2 JQ904072
J
BTV-15 JQ086222
100
K
BTV-27/FRA2014/v01 KM200718
BTV-27/FRA2014/v03 KU760998
BTV-27/FRA2014/v02 KU760988
BTV-X ITL2015
BTV-25 EU839840
100
90
100
L
BTV-26 HM590642
94
100
95
98
82
0.5

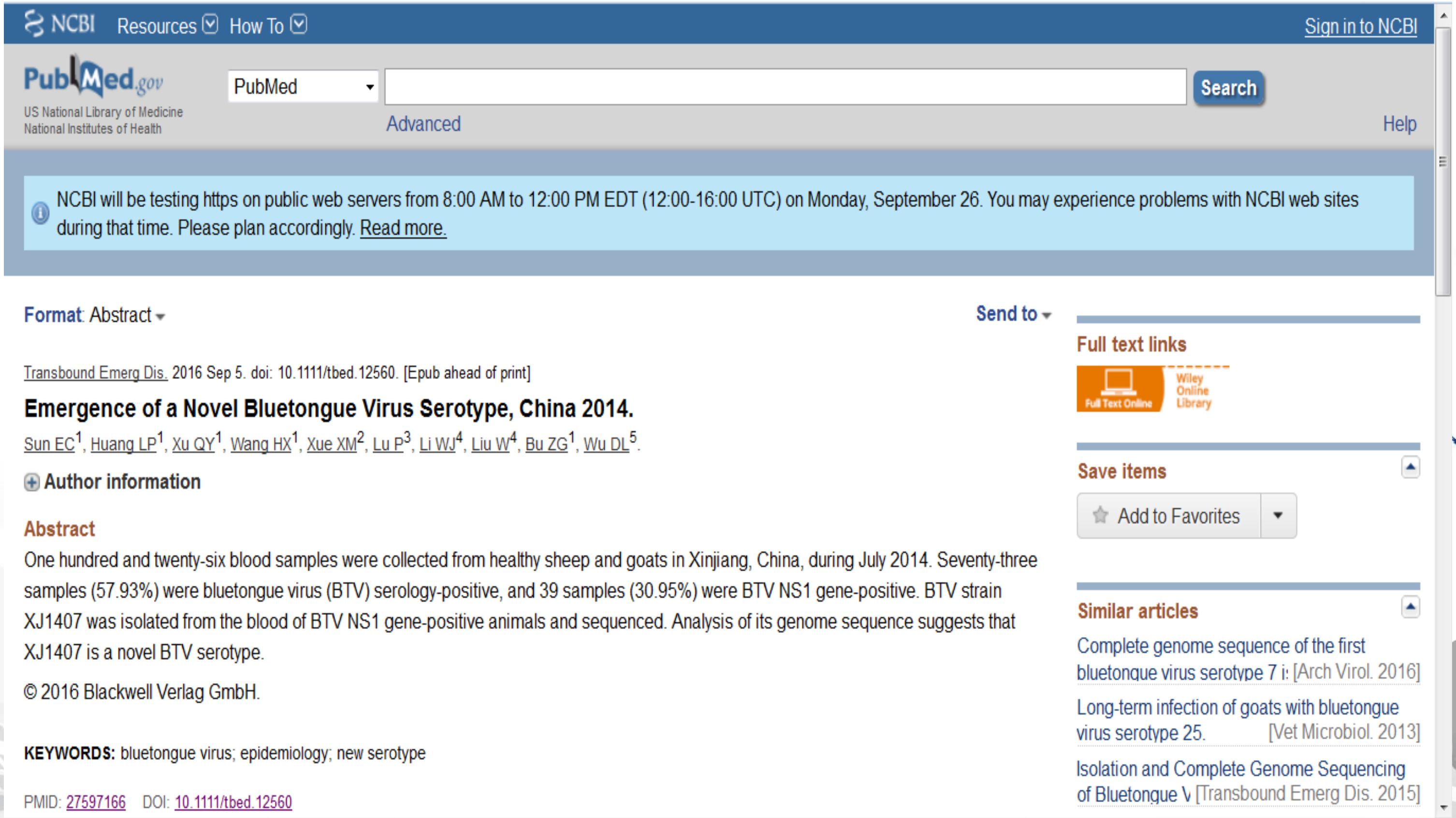
Seg-6


J
BTV-27/FRA2014/v03 KU761002
BTV-27/FRA2014/v02 KU760992
BTV-X ITL2015
100
I
BTV-26 JN255159
100
99
H
BTV-25 EU839842
BTV-27/FRA2014/v01 LN713675
100
B
BTV-14 JX272484
BTV-13 JX272494
BTV-6 JX272564
BTV-9 JN255907
BTV-5 JX272574
BTV-3 JQ086286
BTV-21 JX272414
BTV-16 KF387526
99
BTV-20 JQ086256
BTV-10 JX272524
BTV-17 JX272454
BTV-11 JQ972856
BTV-24 JX272374
BTV-4 JN255887
100
83
92
95
A
78
89
92
C
BTV-23 JX272404
BTV-2 AJ586696
BTV-1 JX861493
100
BTV-18 JX272444
BTV-8 AM498056
G
100
BTV-19 JX272434
BTV-7 JQ086296
D
100
100
BTV-22 JX272394
BTV-12 JX272504
BTV-15 JQ086226
E
F
0.2

BTVX ITL2015



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



NCBI will be testing https on public web servers from 8:00 AM to 12:00 PM EDT (12:00-16:00 UTC) on Monday, September 26. You may experience problems with NCBI web sites during that time. Please plan accordingly. Read more.

Format: Abstract

Send to

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.

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KEYWORDS: bluetongue virus; epidemiology; new serotype

PMID: 27597166 DOI: 10.1111/tbed.12560

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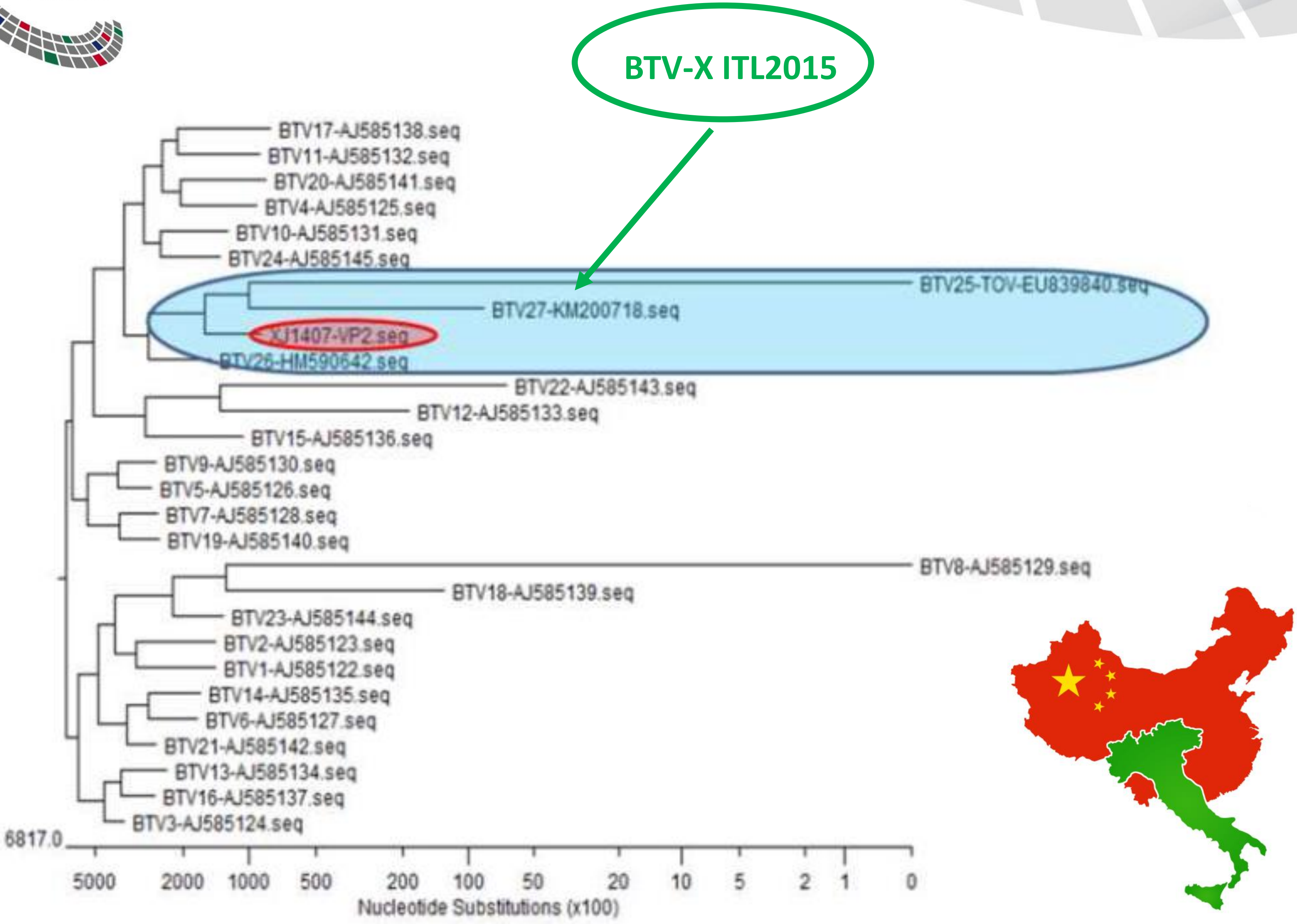
Long-term infection of goats with bluetongue virus serotype 25. [Vet Microbiol. 2013]

Isolation and Complete Genome Sequencing of Bluetongue V [Transbound Emerg Dis. 2015]



BTV-X ITL2015

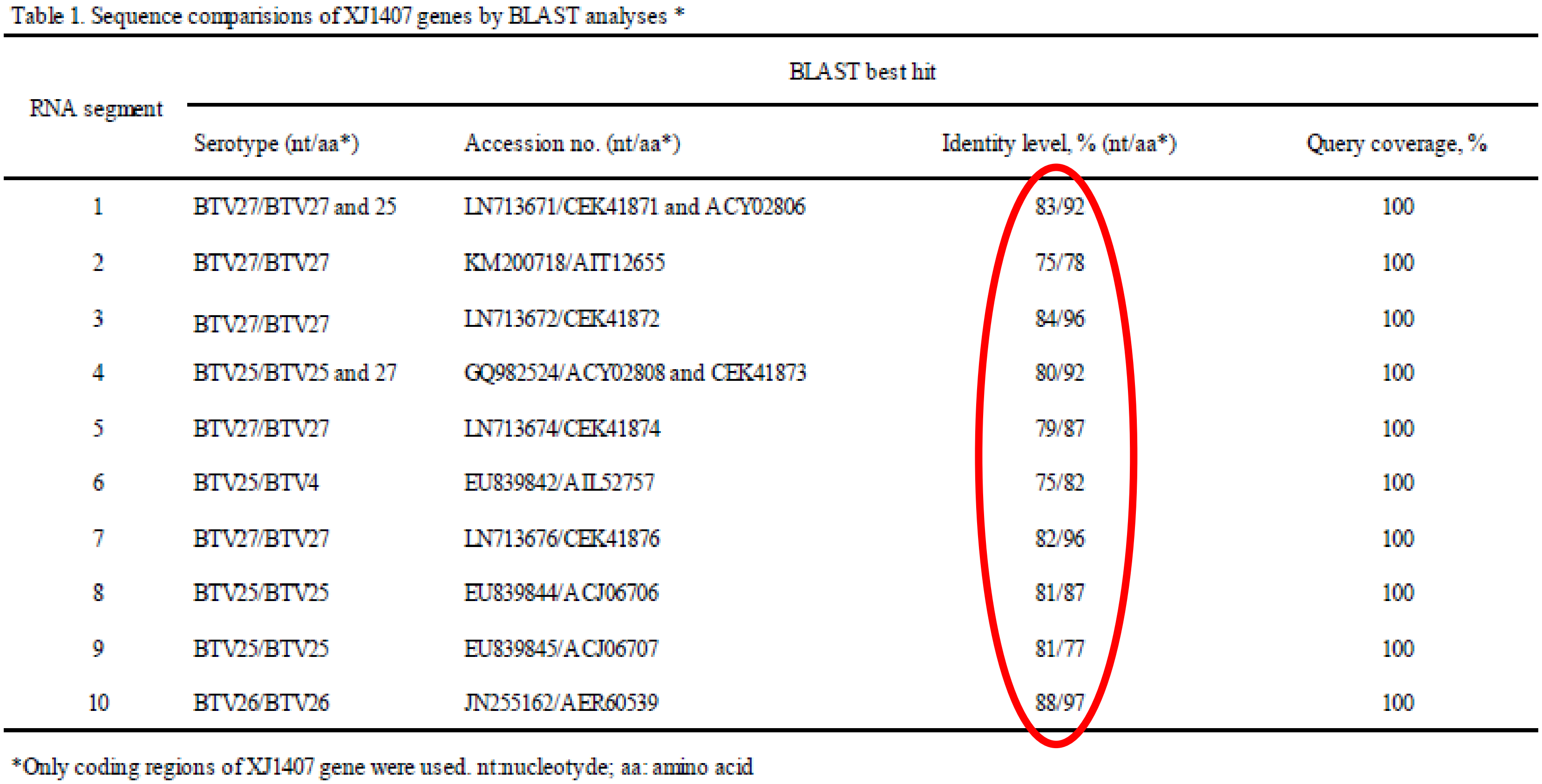


Table 1. Sequence comparisions of XJ1407 genes by BLAST analyses *

RNA segment	BLAST best hit			
	Serotype (nt/aa*)	Accession no. (nt/aa*)	Identity level, % (nt/aa*)	Query coverage, %
1	BTV27/BTV27 and 25	LN713671/CEK41871 and ACY02806	83/92	100
2	BTV27/BTV27	KM200718/AIT12655	75/78	100
3	BTV27/BTV27	LN713672/CEK41872	84/96	100
4	BTV25/BTV25 and 27	GQ982524/ACY02808 and CEK41873	80/92	100
5	BTV27/BTV27	LN713674/CEK41874	79/87	100
6	BTV25/BTV4	EU839842/AIL52757	75/82	100
7	BTV27/BTV27	LN713676/CEK41876	82/96	100
8	BTV25/BTV25	EU839844/ACJ06706	81/87	100
9	BTV25/BTV25	EU839845/ACJ06707	81/77	100
10	BTV26/BTV26	JN255162/AER60539	88/97	100

*Only coding regions of XJ1407 gene were used. nt:nucleotyde; aa: amino acid

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

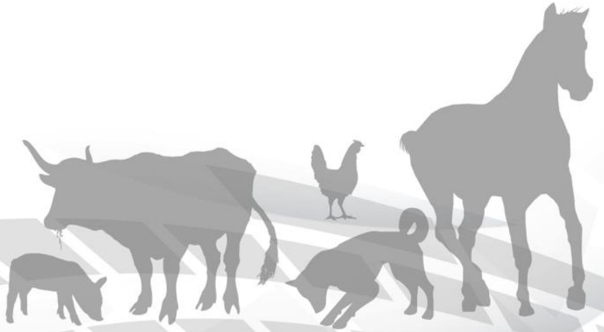
- The partial VP2 sequence confirmed that these three strains have homologies less than 75% with all other serotypes

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

Conclusions

For the Bluetongue control, keywords are:

- **COLLABORATION**
  - the strict collaboration between different professionalities particularly between field, laboratories and policy makers
    - “**information sharing and constant updating** on what occurring in the field (novel viruses and serotype)”



Conclusions

For the Bluetongue control, keywords are:

- **FEXIBILITY**
  - **Adopt different measures** according to the different masks BTV wears
- **COORDINATION**
  - Any strategy is affordable only if perfectly coordinated