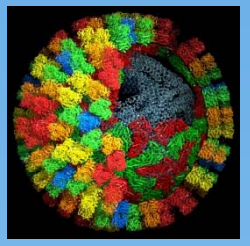


The Emergence of Exotic Bluetongue

the importance of vaccination and future threats

Uwe Mueller-Doblies, Narender Maan, Kyriaki Nomikou, Sushila Maan, and Peter Mertens

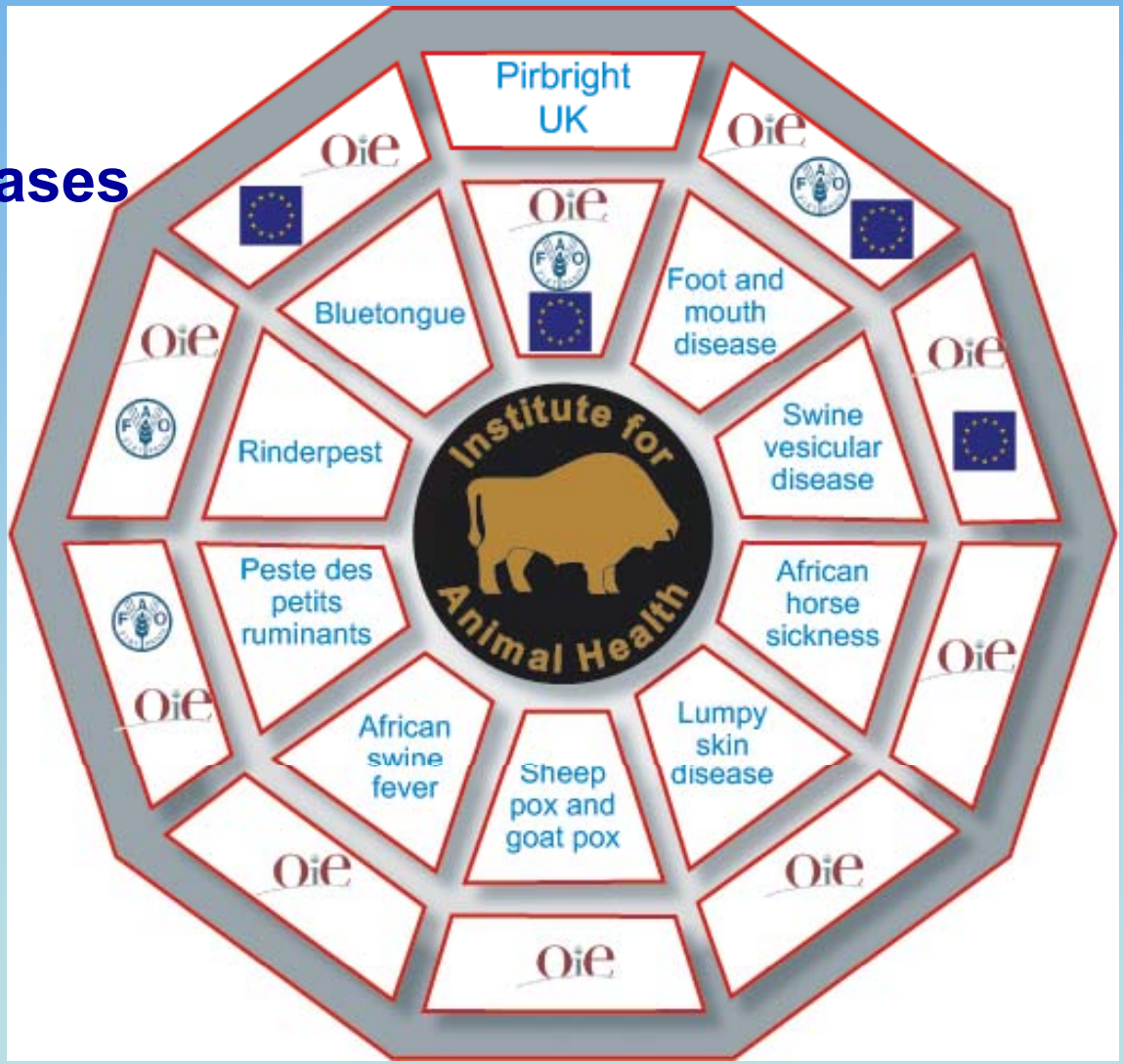
Biosecurity Group and Vector Borne Diseases Programme,
Institute for Animal Health , Pirbright UK.

USDA ARS 1st International Biosafety & Biocontainment Symposium

The reference labs at IAH-Pirbright

Reference labs in Vector-borne Diseases Programme

- Bluetongue
- African Horse Sickness
- Lumpy Skin Disease
- Sheep and Goat Pox
- African Swine Fever



defra Department for Environment Food and Rural Affairs

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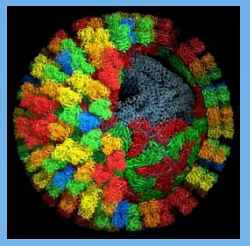
Overview



- Bluetongue Disease
- Blue Tongue Disease Viruses
- Dynamic of recent emergence in Europe
- Aspects of Emergence
  - climatic changes
  - vector competency
  - live attenuated vaccines
  - recombination
- Biosafety Issues
-

Overview

- Bluetongue Disease
- Blue Tongue Disease Viruses
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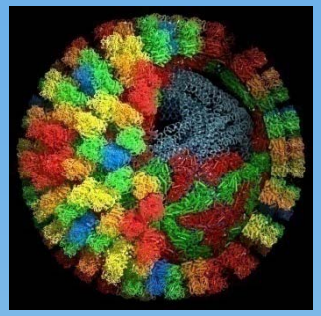

Bluetongue: Clinical signs and disease in Europe

"On a global basis, the second most economically important disease of livestock after Foot and Mouth"

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Coronitis
Conjunctivitis
G. BOSQUET SNGTV
G. BOSQUET SNGTV
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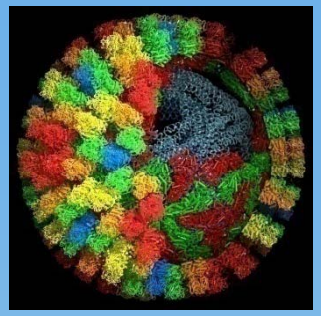
: Dummy calf at pasture 1b - Williamson, et al Vet Record 20


4-week old calf with behavioural & locomotory problems.
Brain with cerebral hemispheres replaced by a fluid filled sac. (Wouda et al 2008)



Bluetongue virus:
Classification and distribution

Institute for Animal Health

Classification of *Orbivirus* species (serogroups)

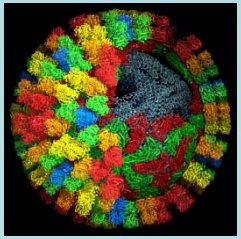
1-*African horse sickness virus* (AHSV)
2-***Bluetongue virus (BTV)***
3-*Changuinola virus* (CGLV)
4-*Chenuda virus* (CNUV)
5-*Chobar Gorge virus* (CGV)
6-*Corriparta virus* (CORV)
7-*Epizootic hemorrhagic disease virus* (EHDV)
8-*Equine encephalosis virus* (EEV)
9- *Eubenangee virus* (EUBV)
10-*Ieri virus* (IERIV)
11- *Great Island virus* (GIV)
12- *Lebombo virus* (LEBV)
13-*Orungo virus* (ORUV)
14-*Palyam virus* (PALV)
15- *St Croix River Virus* (SCRV)
16-*Umatilla virus* (UMAV)
17-*Wad Medani virus* (WMV)
18-*Wallal virus* (WALV)
19-*Warrego virus* (WARV)
20-*Wongorr virus* (WGRV)
21- *Peruvian horse sickness virus* (PHSV)
22- *Yunnan Orbivirus (YUOV)*

Total: 22 virus species, 161 serotypes

Tentative species / unassigned viruses

Andasibe virus (ANDV)
Ife virus (IFEV)
Itupiranga virus (ITUV)
Japanaut virus (JAPV)
Kammavanpettai virus (KMPV)
Lake Clarendon virus (LCV)
Matucare virus (MATV)
Tembe virus (TMEV)
Codajas virus (COV)
Tracambe virus (TRV)

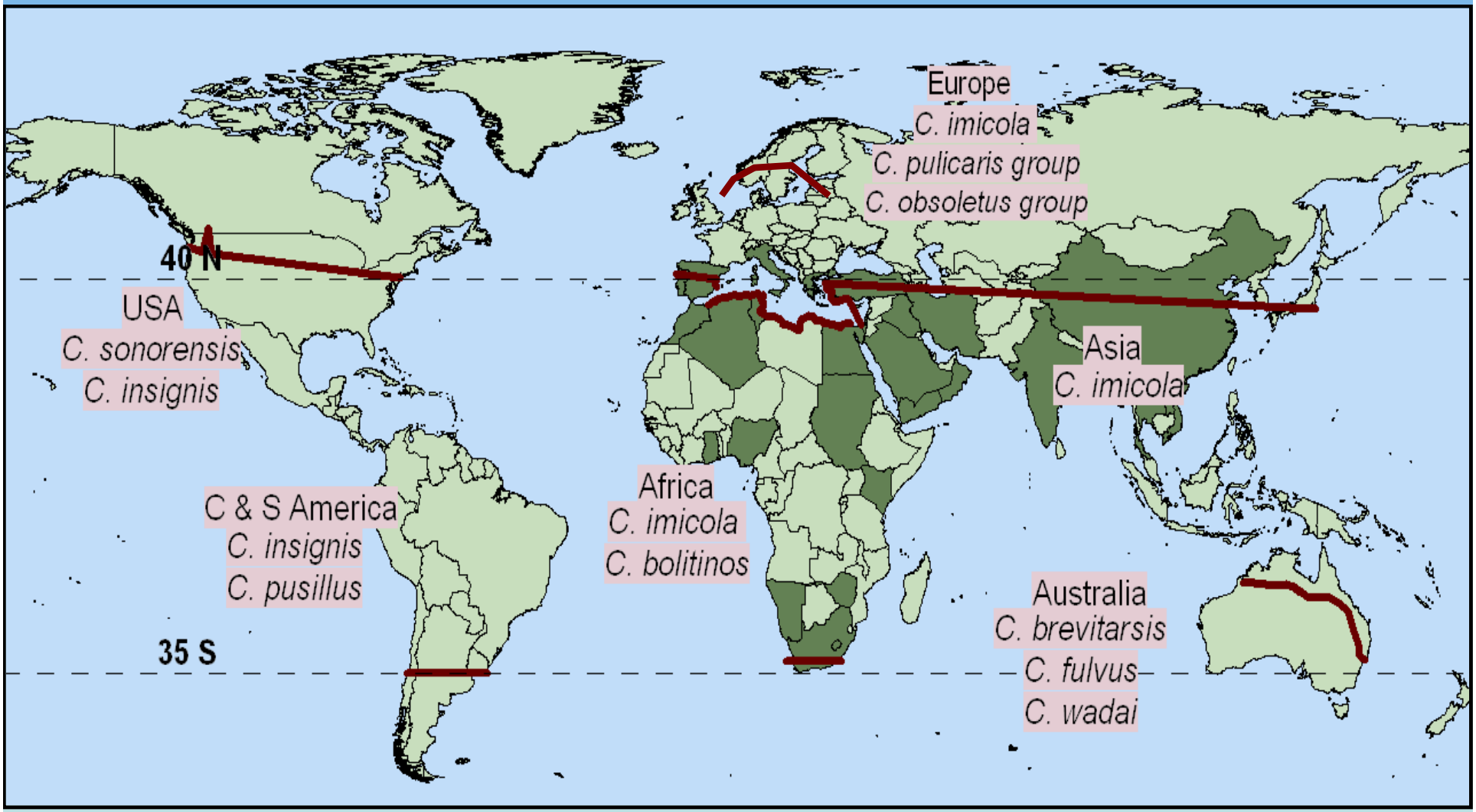
Total : 12 unassigned viruses



Global distribution of the 25 Bluetongue virus serotypes

Region	Bluetongue virus serotype (I = virus serotype isolated: S = serological evidence for each serotype (detection of neutralising antibodies)																								
-	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Africa	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	-	I	I	-	I	-	I	-	-
Middle East	-	I	-	I	-	I	-	-	S	I	-	S	S	S	I	I	S	-	S	S	-	-	-	I	-	**I**
India + Pakistan	I	I	I	I	S	I	S	S	I	S	S	S	S	S	S	I	I	I	-	S	S	-	I	-	-	-
Australia	I	I [6]	I	-	-	-	**I [4]**	-	I	-	-	S	-	-	I	I	-	-	-	I	I	-	I	-	-	-
South East Asia and Indonesia	I	I	I	I	-	-	I	-	I	I	S	I	S	-	-	S	I	S	S	S	I	-	I	-	-	-
China	I	I	I	I	-	-	-	-	-	-	-	I	-	-	I	I	-	-	-	-	-	-	-	-	-	
USA	I	I [1,2]	I	-	I	I	-	-	-	I	I	-	I	I	-	-	I	-	I	-	-	I	-	I	-	-
Central and South America	I	-	I	I	-	I	-	I	-	-	I	I	I	I	-	S	I	-	-	-	-	-	-	-	-	-
Europe	I*	I*	I	I*	-	I*	-	I* [5]	I*	I	R		-	-	-	I*	-	-	-	-	-	-	-	-	**I***	-

The global distribution of Bluetongue virus (pre 2006)



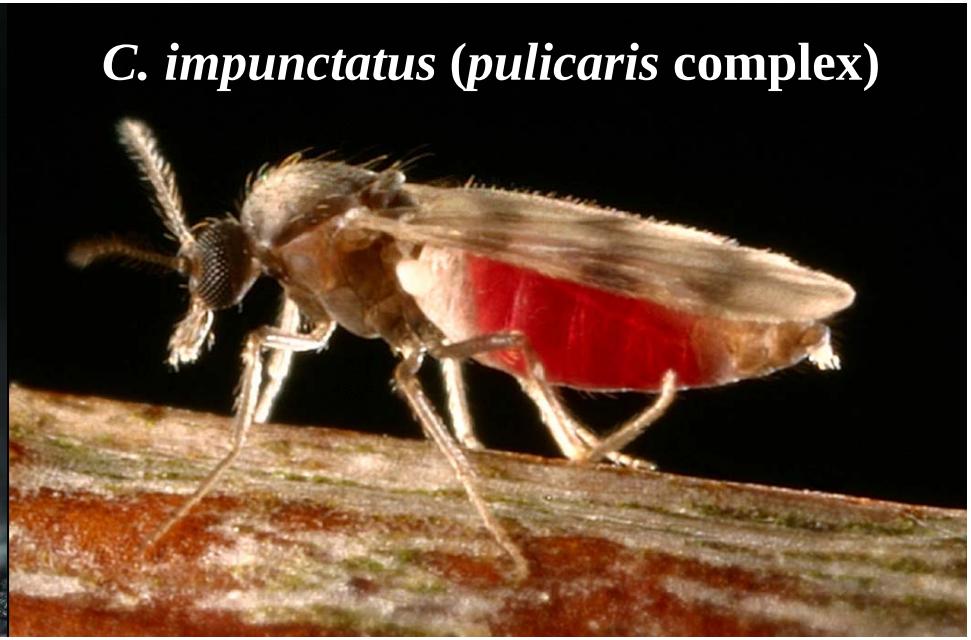
Culicoides vector species from Europe, Africa and the USA

C. obsoletus

C. sonorensis

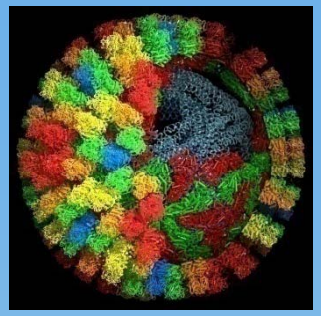
C. imicola

C. impunctatus (*pulicaris* complex)



Adult female *Culicoides nubeculosus* (from the insect colony at IAH Pirbright)

~1mm

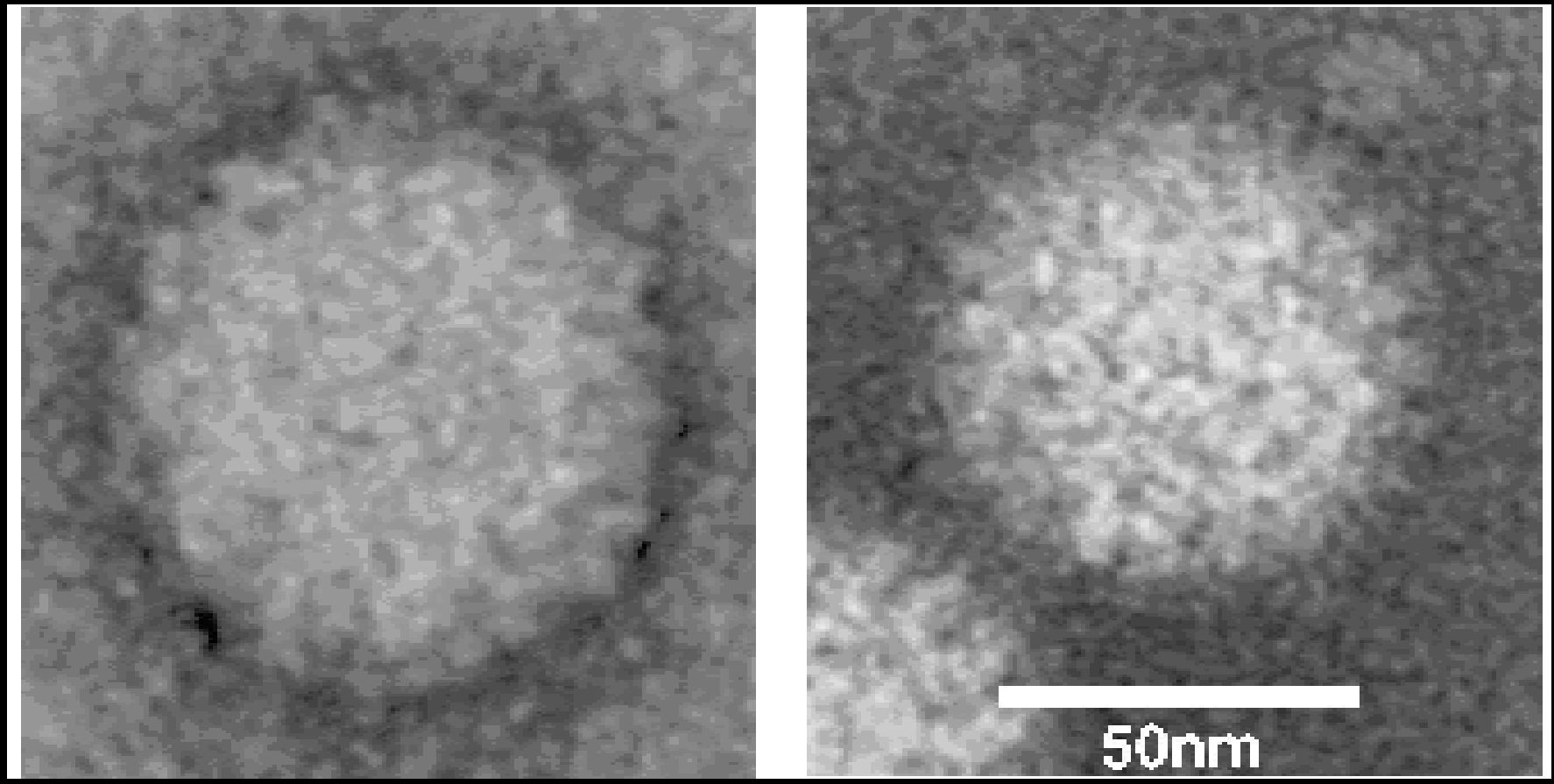


Bluetongue virus:
Structure and serological properties

Institute for Animal Health

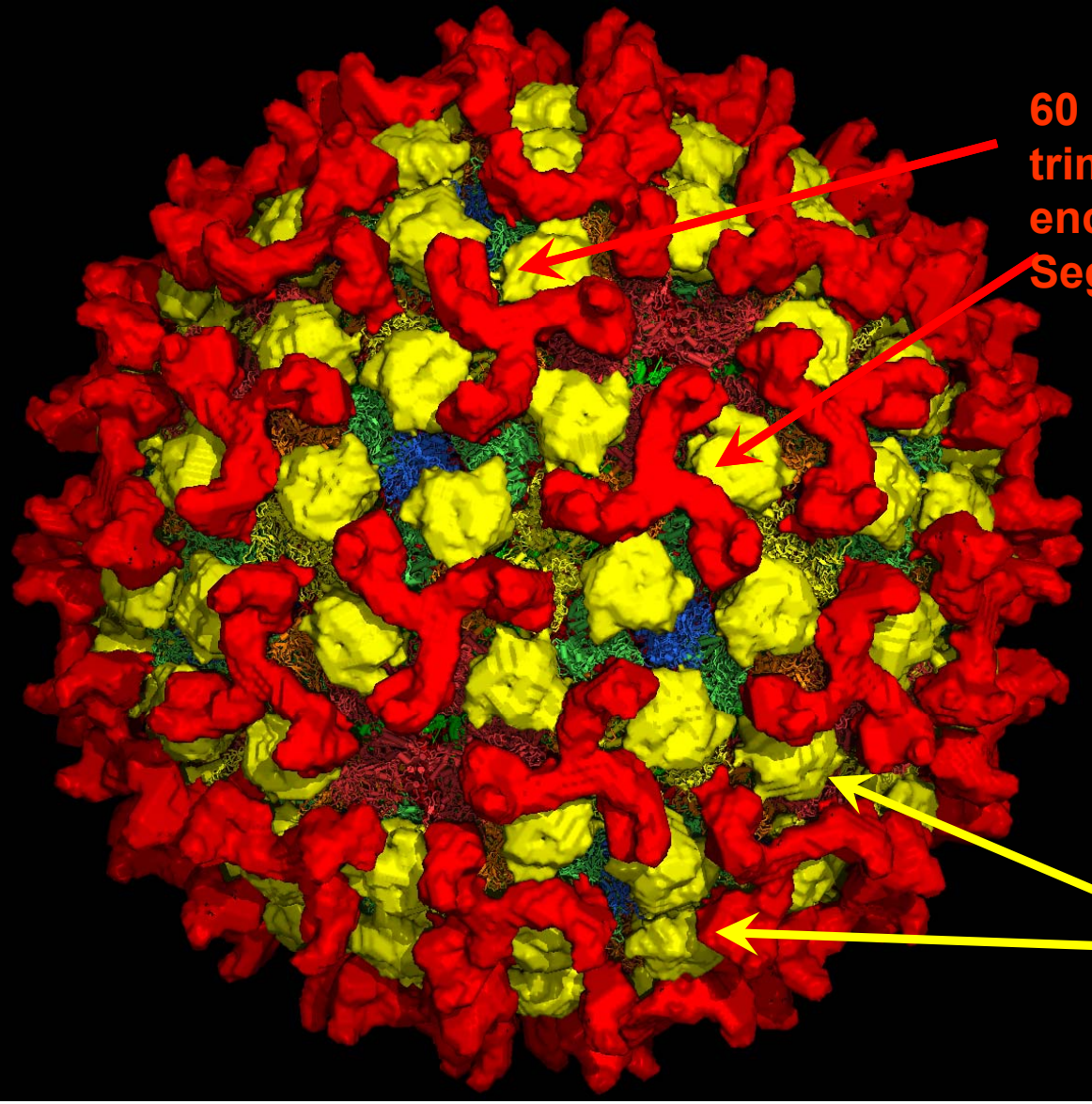
BTV-1
Virus particle

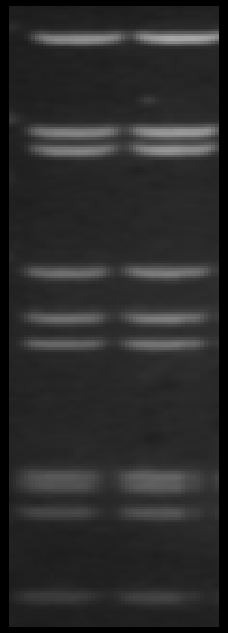
BTV-1
Core particle



Assembly of the major bluetongue virus capsid proteins

The outer surface of the BTV virus particle
VP and VP5 - Encoded by segments 2 and 6
(Cryo-EM and x-ray crystallography reconstruction)

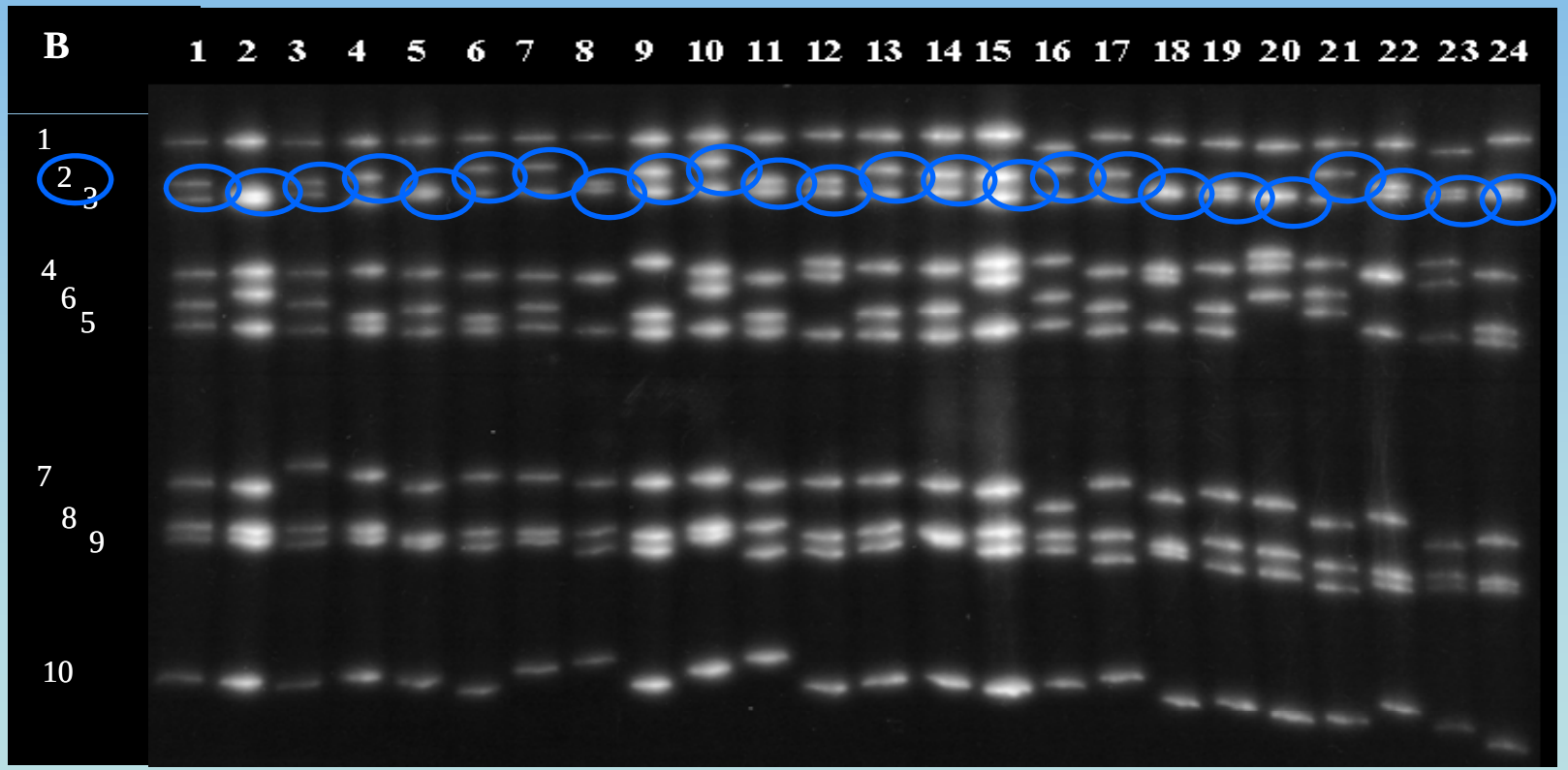




60 VP2 trimers encoded by Seg-2

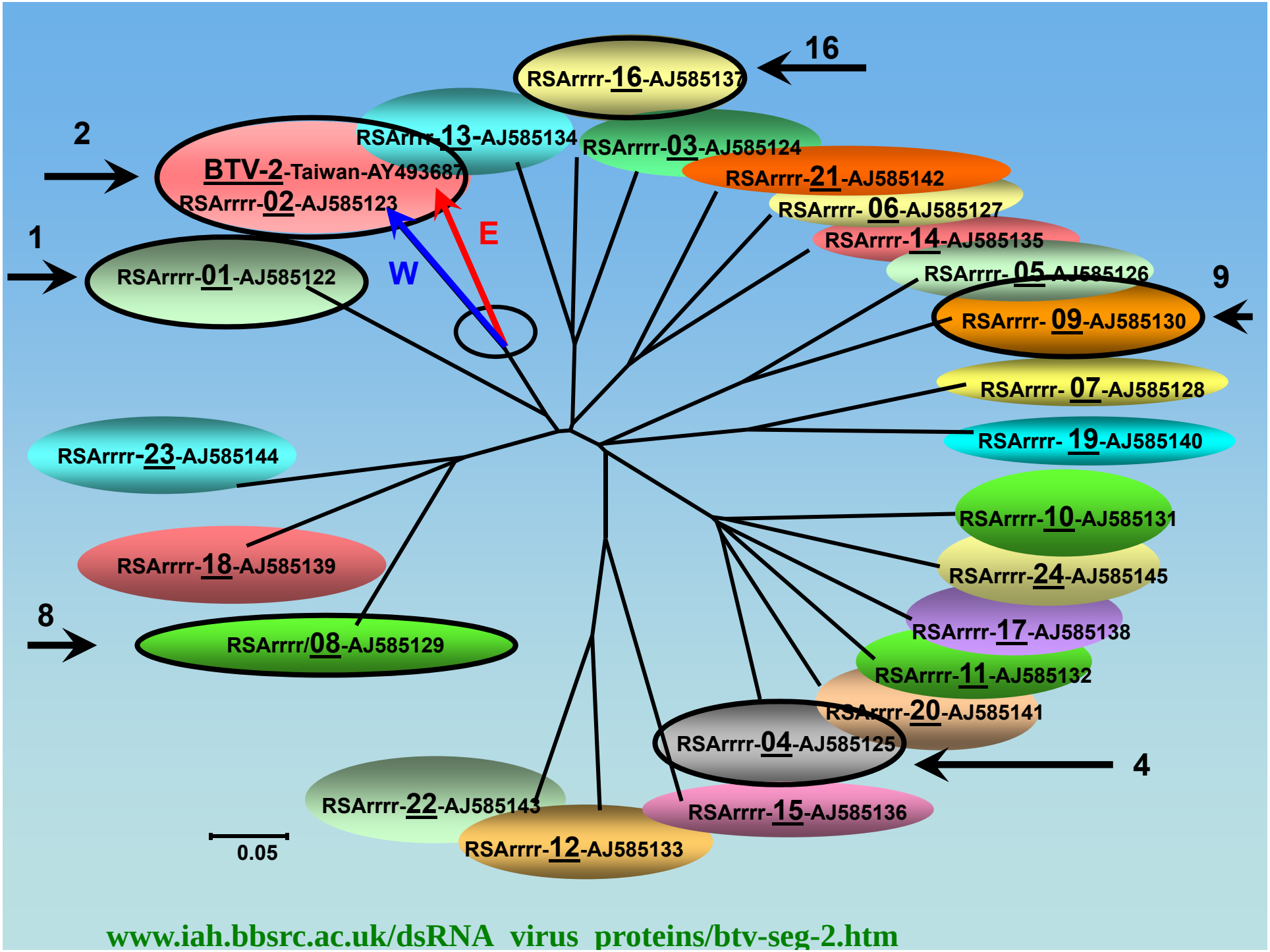
120 VP5 trimers encoded by Seg-6

The Genome segments of the 24 BTV serotypes analysed by SDS-PAGE

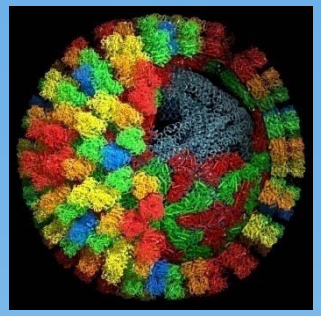


Primers for identification of European BTV serotypes by Rt-PCR since1998

Type	FWD Primer	RVS Primer	Product BP)
1	BTV-1/2/p406F GAGAACARGAARAATATAT	BTV-1/2/p968R TYATACGTTGAGAAGTTTTGT	1686 (1A)
2	BTV-2/2/p198F TATCAGTTAATAGTGCATT	BTV-2/2/p416R TACTAAAKATATACTTCTCCGT	653 (2A)
3C	BTV-3/2/p340F GAACTAATGAGGACAGAT	BTV-3/2/p840R TGAGATCGTCGTCTGAAGAT	1500
4	BT4/67/F GCTTAACTATAAACCAACGAGG	BT4/775/R GTATCAACCTGACCGCGTCG	2124
5	5NIG/123/F TACGGATCATAAATGGATGG	5NIG/791/R TATATGCTCACAGATATCT	2004
6	BT6/301/F GGTGGTATGTATAGAGGAAG	BT6/853/R CAAAGGGAACCTCGCGCGTAATC	1656
7	BT7/83/F GATGTATCCATAGCGGCAT	BT7/853/R GATAGATAAGCGAATCGAG	2310
8	BT8/101/F GAATGATGGTCATAGCGAG	BT8/500/R CGATGTGCGCATTCCTCTC	1197
9	BTV-9/2/p491F GGSAATATATTTCTRATGG	BTV-9/2/p867R GGAAAACTGATTGGCAAT	1128 (9C)
10	BT10/183/F GATGTTCCACATCTTACAG	BT10/803/R GTGAAACCTAATGAAATTG	1860
11	BT11/147/F TGTATTGTTAAGGCTAGG	BT11/770/R GTCATCGTATAGTATCAT	1569
12	BT12/111/F GGATCACAATATAGATGTG	BT12/776/R CTACGATCATATGATAACTC	1995
13A	BT13/157/F CGAGGAAAGCGGATACCAC	BTV-13/2/p631R TGCTTAATGCTCAAACTCG	1422
14	BT14/121/F GAAGGTTAGCTTAGGTTTG	BT14/805/R CTCCGCTTCATCCAGCTC	2052
15	BT15/343/F GTGGCAGAACGCAGAGGCAG	BT15/806/R G TGAGACATATAATGTTCAAG	1389
16B	BTV-16/2/p266F GCGATAAGAGATAMTTGGAT	BTV-16/2/p924R GCCGAAGGTGCGATCTGGCCG	1974 (-Nig)
17	BTV-17/2/p146F GCGAATGCTGCCAACGCTG	BTV-17/2/p653R GCGCGACTTTCCTAAACG	1521
18	BT18/160/F GTCTTATCATATAGAGCCAG	BT18/828/R GTACTCAGATAATAGTCGAG	2004
19	BT19/190/F TGTGCTCAAGCAAGCGCGTAT	BT19/841/R GTCATCGTCGAGTGCGTG	1953
20	BT20/135/F CATTACTCGATAGATTACC	BT20/812/R GTACGTCGTCAGCAATCTG	2031
21	BT21/116/F GAGGAATGGCTGAACTGG	BT21/754/R GCCTCCACACAGCGAAGACAG	1914
22	BTV-22/311F GACGCGTTGGATAGGATAT	BTV-22/682R GCTTATACCTCGCATCATCG	1113
23	BT23/459/F GCTTAGACCTGGCGATAAG	BT23/653/R GTTTAACATGCATACTCAG	582
24	BT24/313/F TGGATTTATCTACACGATT	BT24/783/R CATAAGCTCCAACTTCAAC	1410



www.iah.bbsrc.ac.uk/dsRNA virus proteins/btv-seg-2.htm



BT in Europe 1998 to 2006:
Transmission and spread

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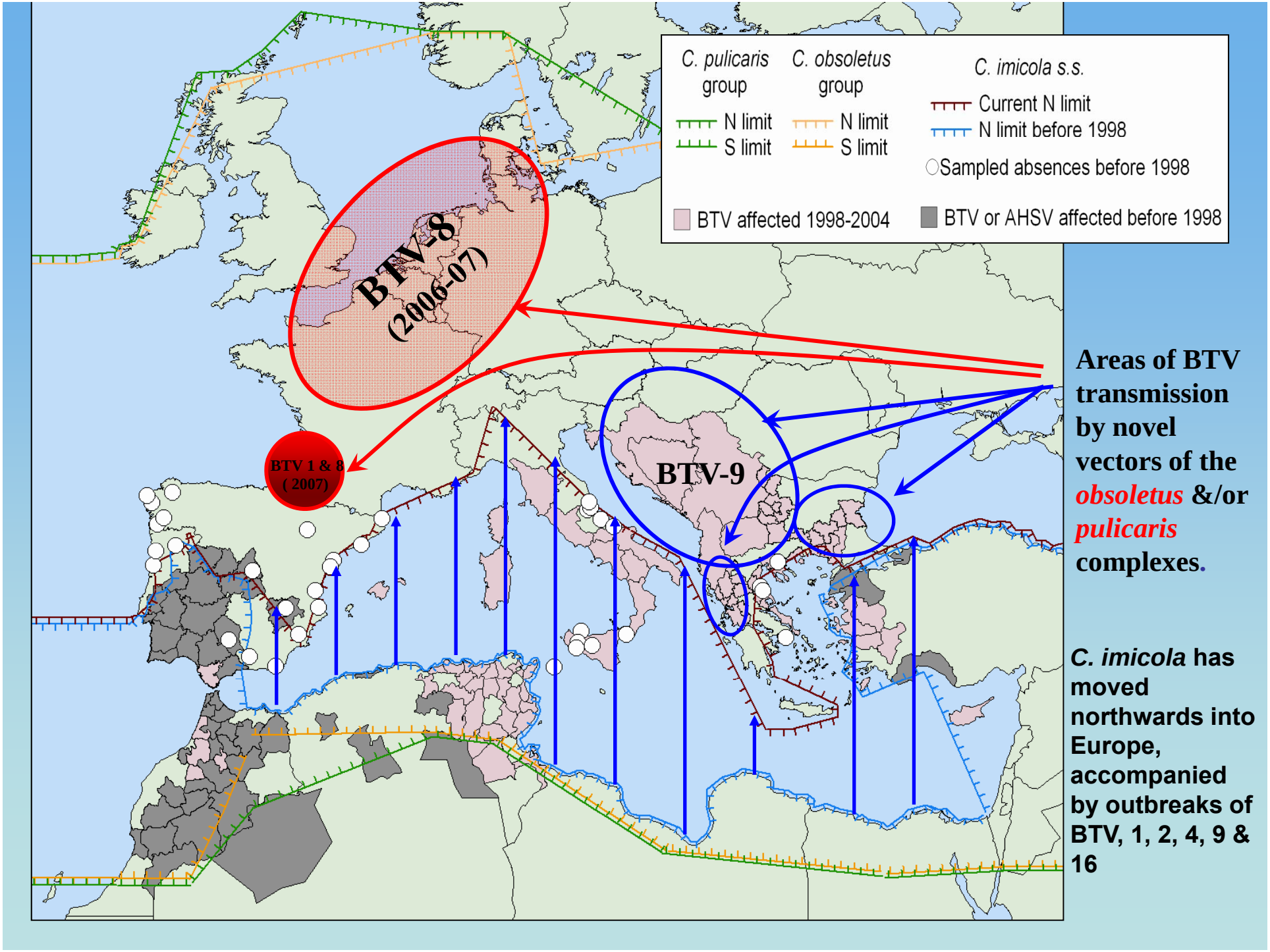
Northernmost Distribution of BTV to 1997

BTV-10 in Spain & Portugal 1956 - 60, BTV-4 in Turkey and Cyprus during 1980's .
BTV-3 in Lesbos and Rhodes 1979.

Northern most distribution of *Culicoides imicola* (to 1997)

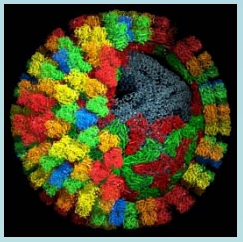


Areas of BTV transmission by novel vectors of the *obsoletus* &/or *pulicaris* complexes.

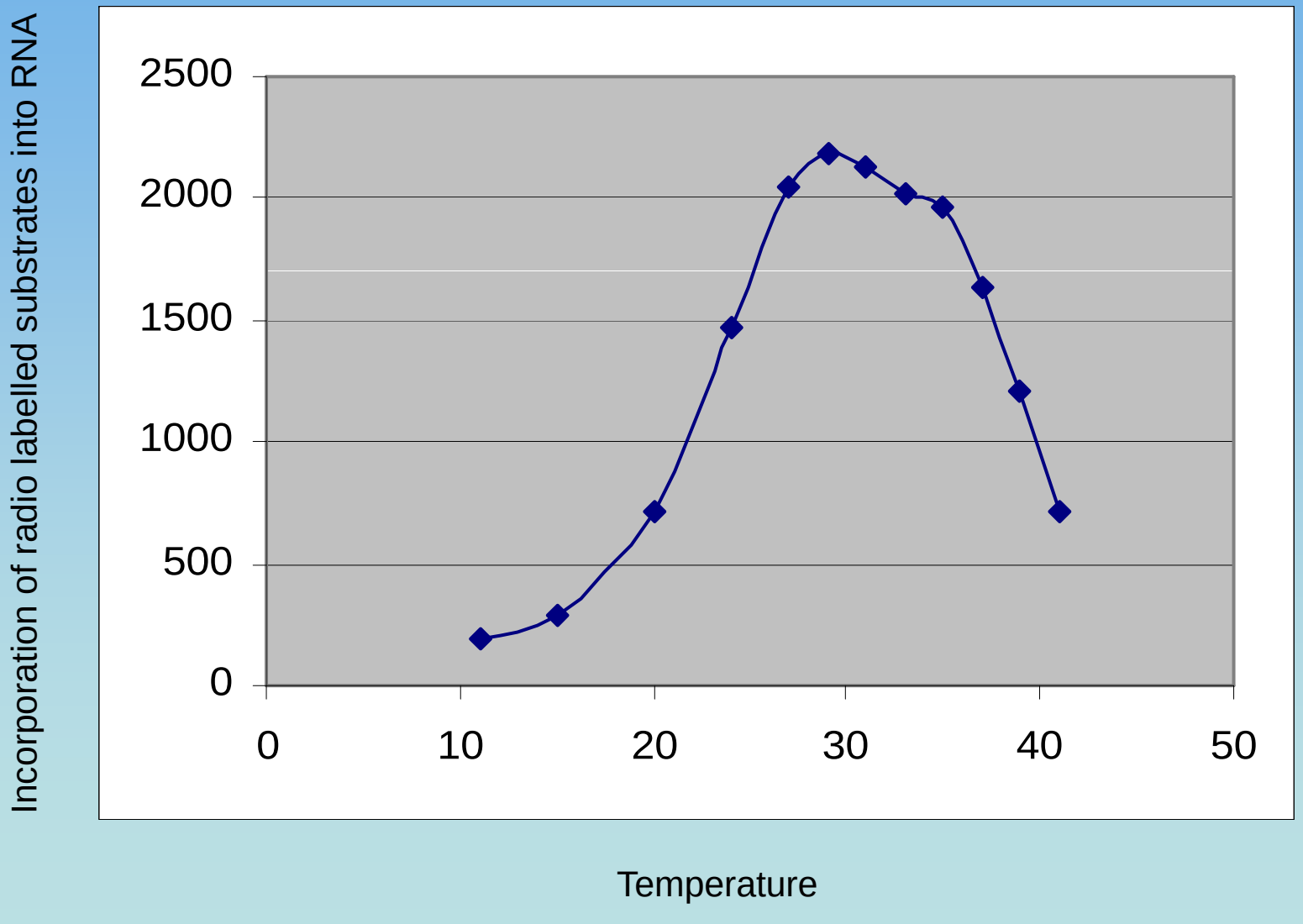
***C. imicola* has moved northwards into Europe, accompanied by outbreaks of BTV, 1, 2, 4, 9 & 16**

15 distinct strains of nine different BTV serotypes have invaded Europe, with new introductions every year Since 1998

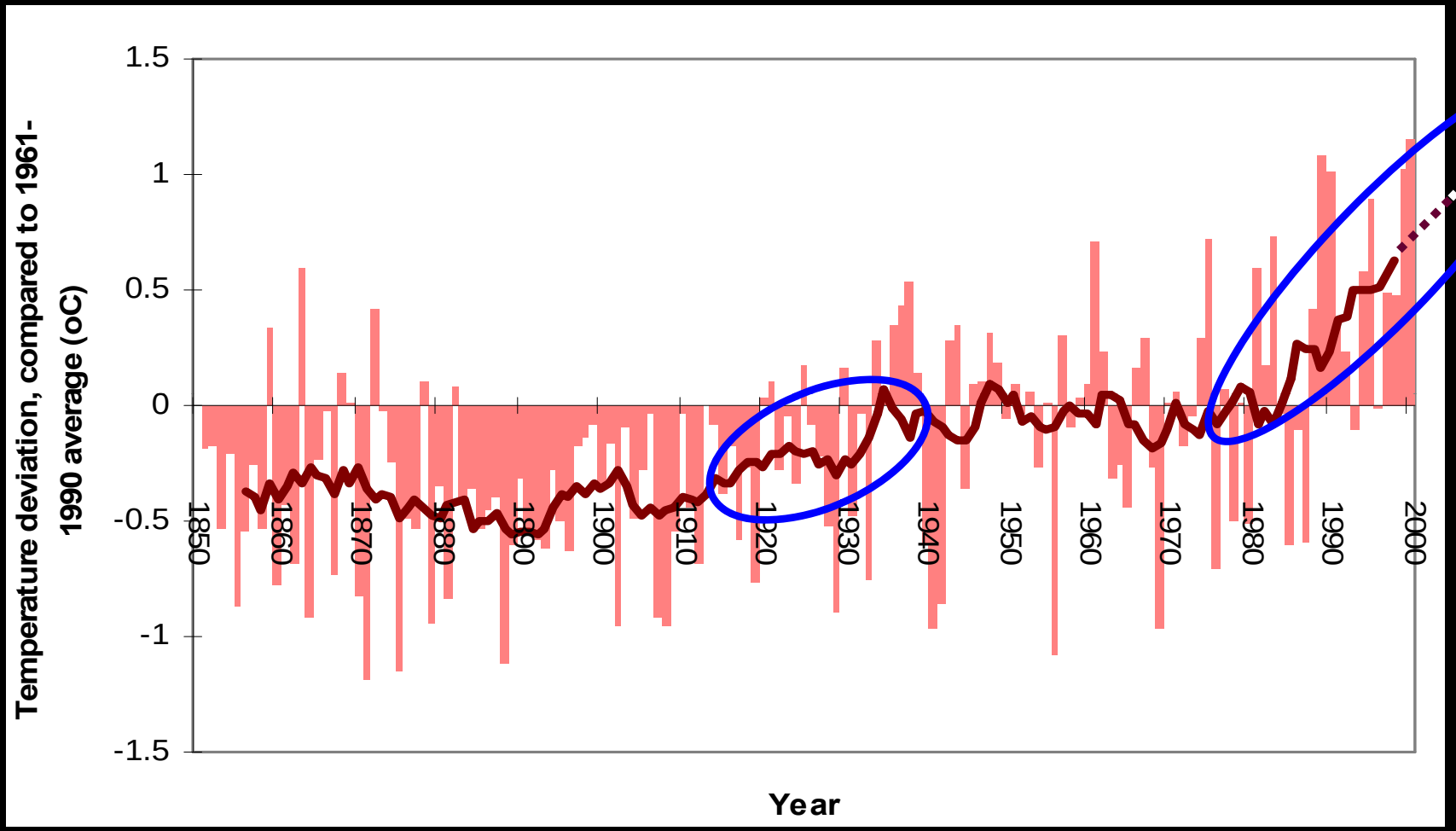
This evidence supports that a fundamental 'step-change' in the epidemiology of BTV that has been related to climate change



BTV -1 polymerase activity



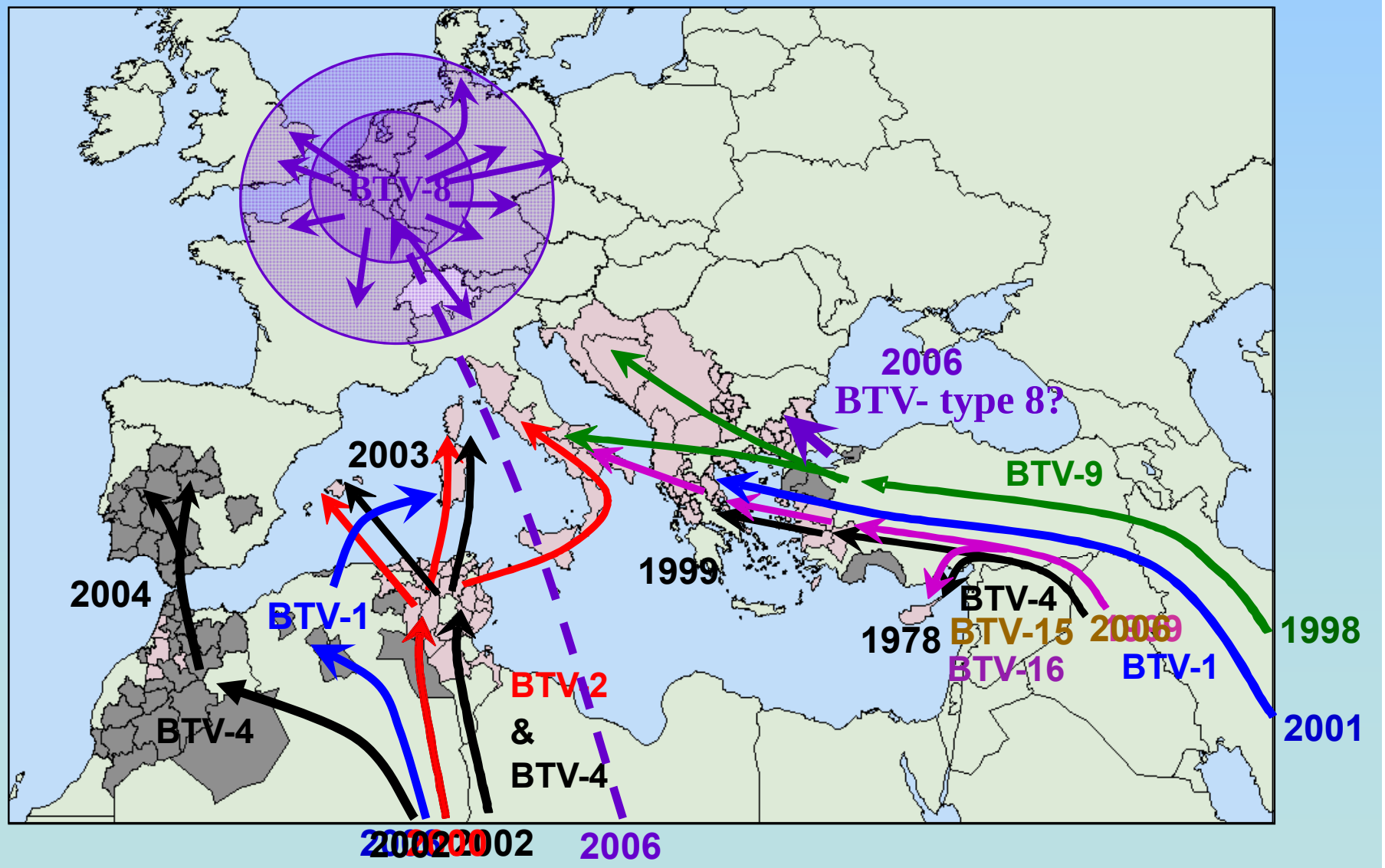
Recent climate warming in Europe.

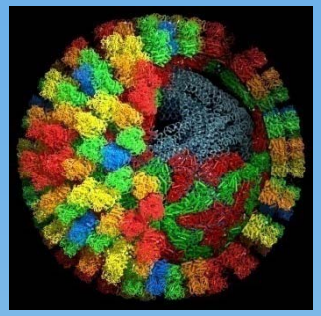


Recent warming trend in Europe. observed annual temperature deviations in Europe over the last 140 years (1850 to 2000) compared to the 1961 to 1990 average (ºC) as light pink bars. Superimposed as a continuous dark pink line is the 10-year smoothed trend of these annual temperature deviations (reproduced with permission from EEA, 2004). (Purse et al 2005 Nature Reviews)

Wind blowing dust from the Sahara across Italy and the Mediterranean Islands

The 10 introductions of different BTV strains into Europe and north Africa 1998 to end of 2006





BTV-8 in Europe: 2006 onwards

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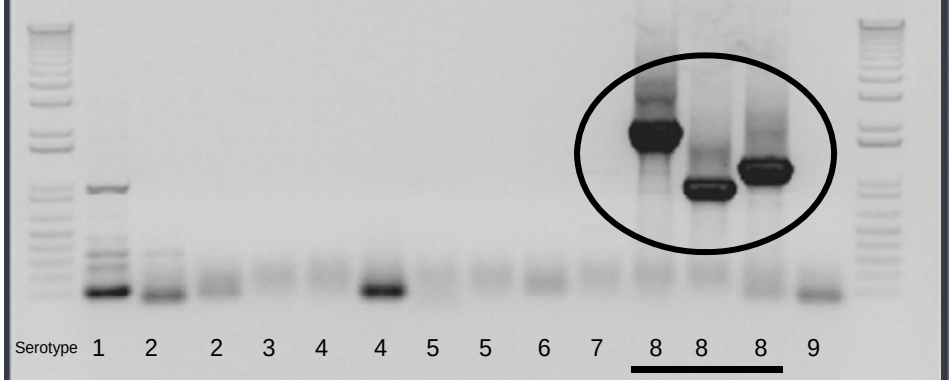
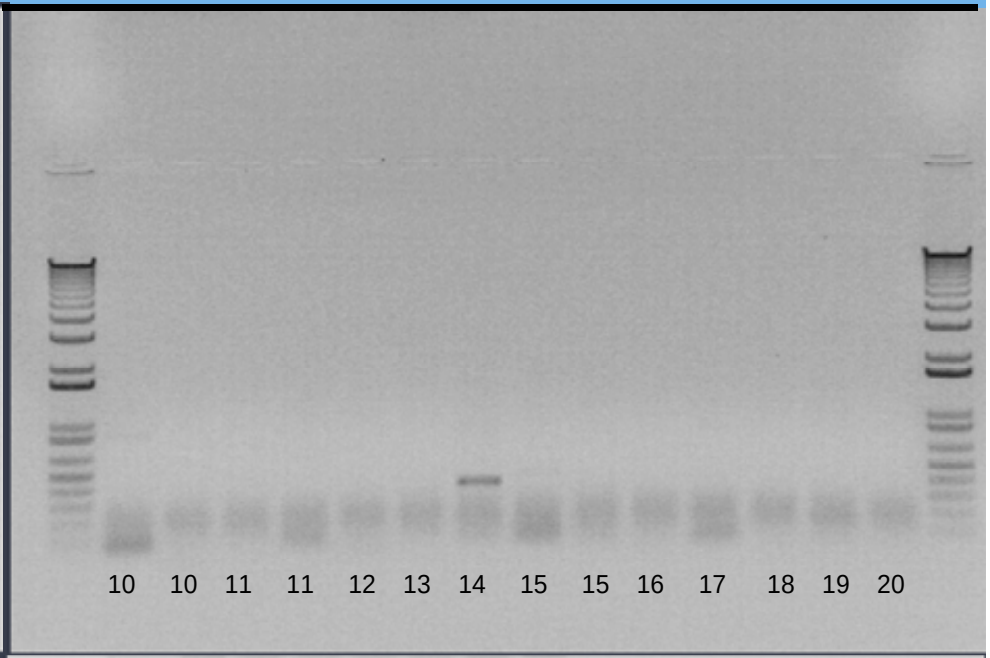
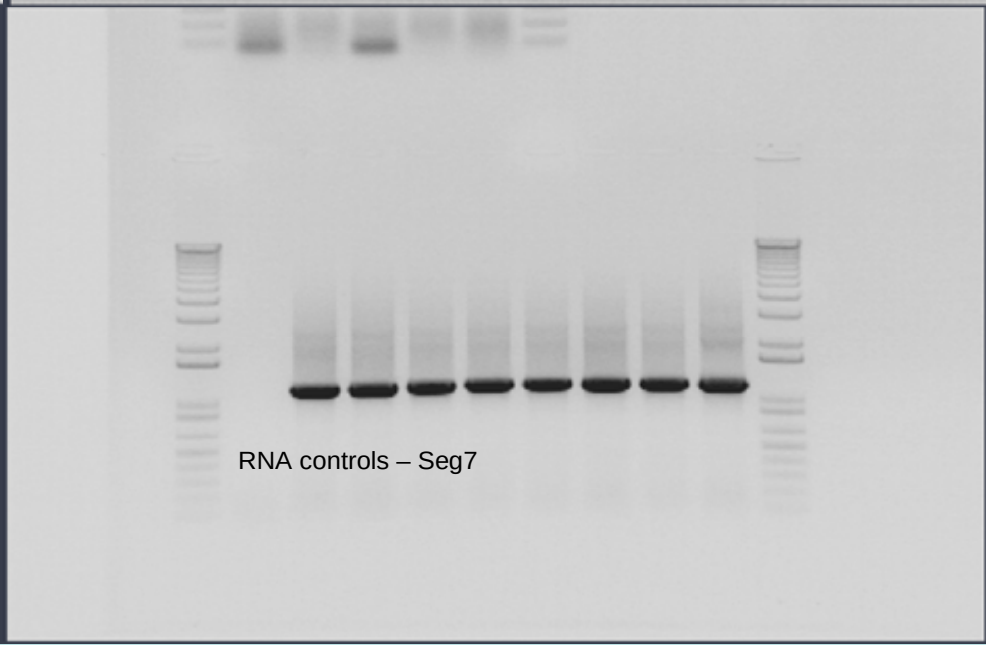
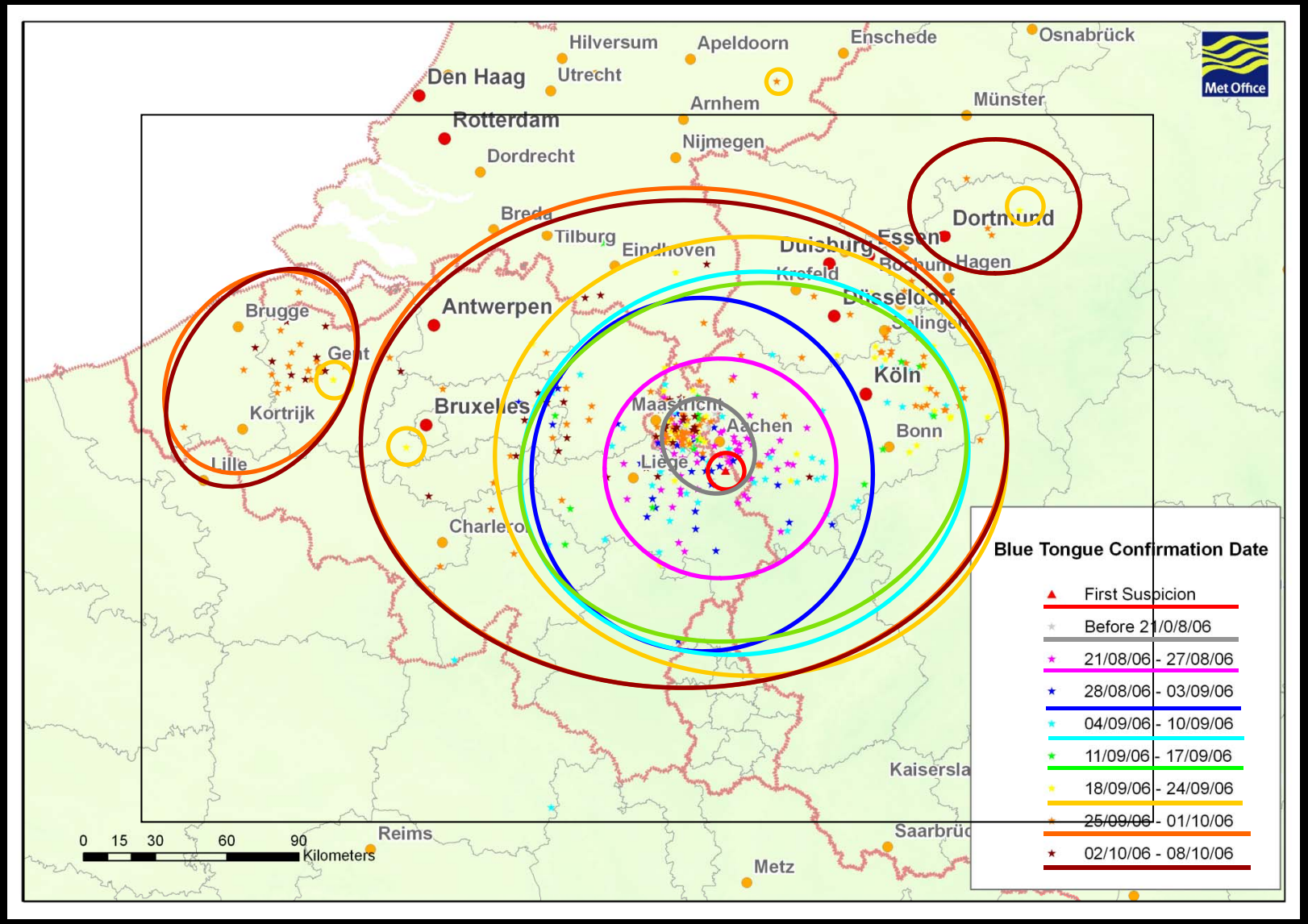
Initial identification of NET2006/01 as BTV-8

Serotyping. Confirmed by typing primers against S2 as being type 8. There is a faint band for BTV1 but it is completely of the wrong size.



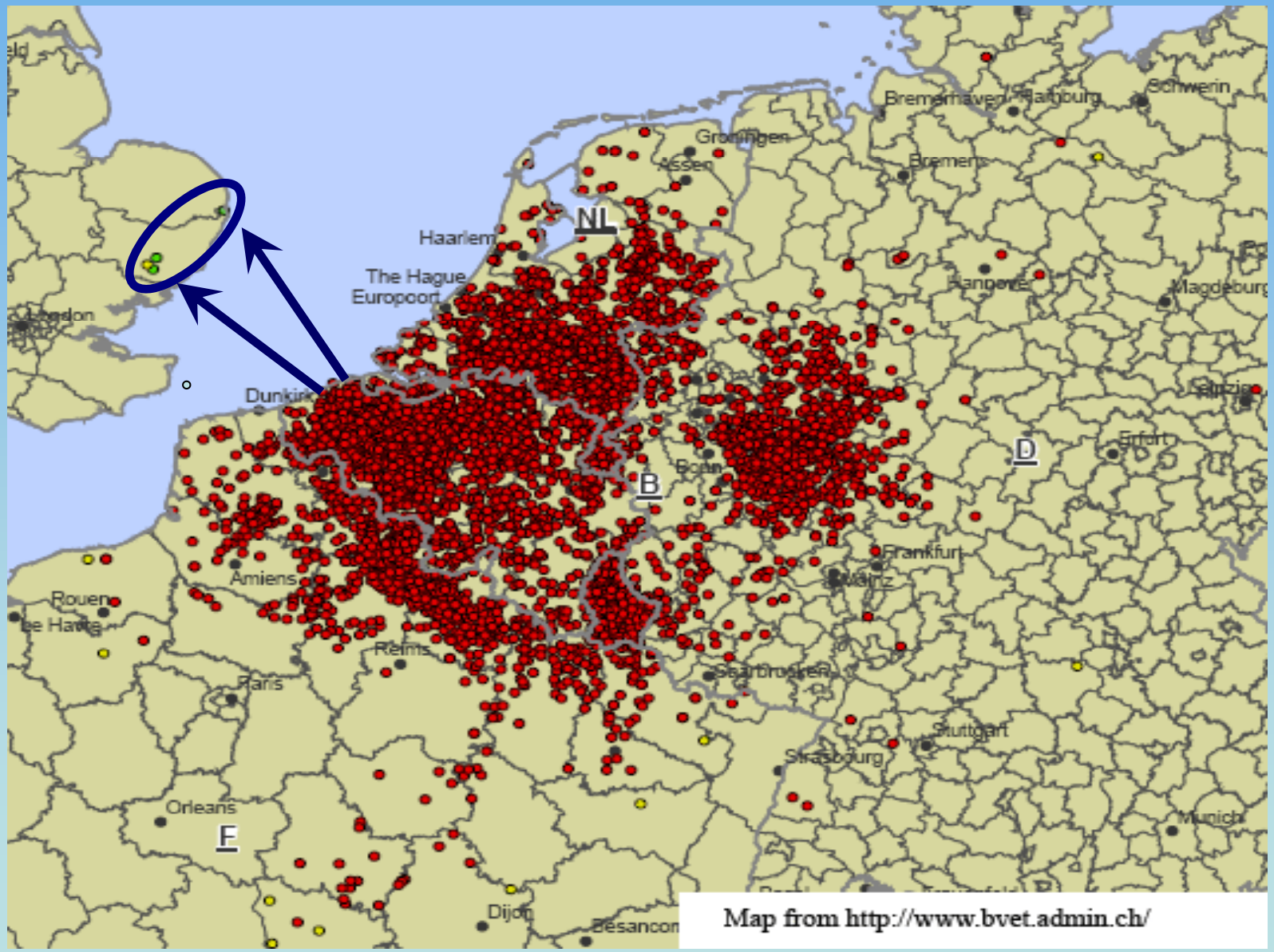


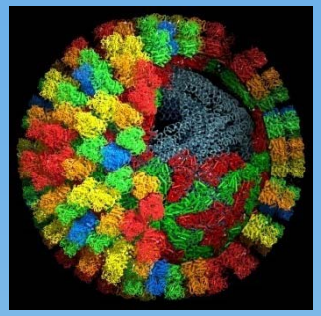




The Spread of BTV outbreaks from 21st of August to 8th October 2006

European BTV situation 2007





BTV-8 in Germany

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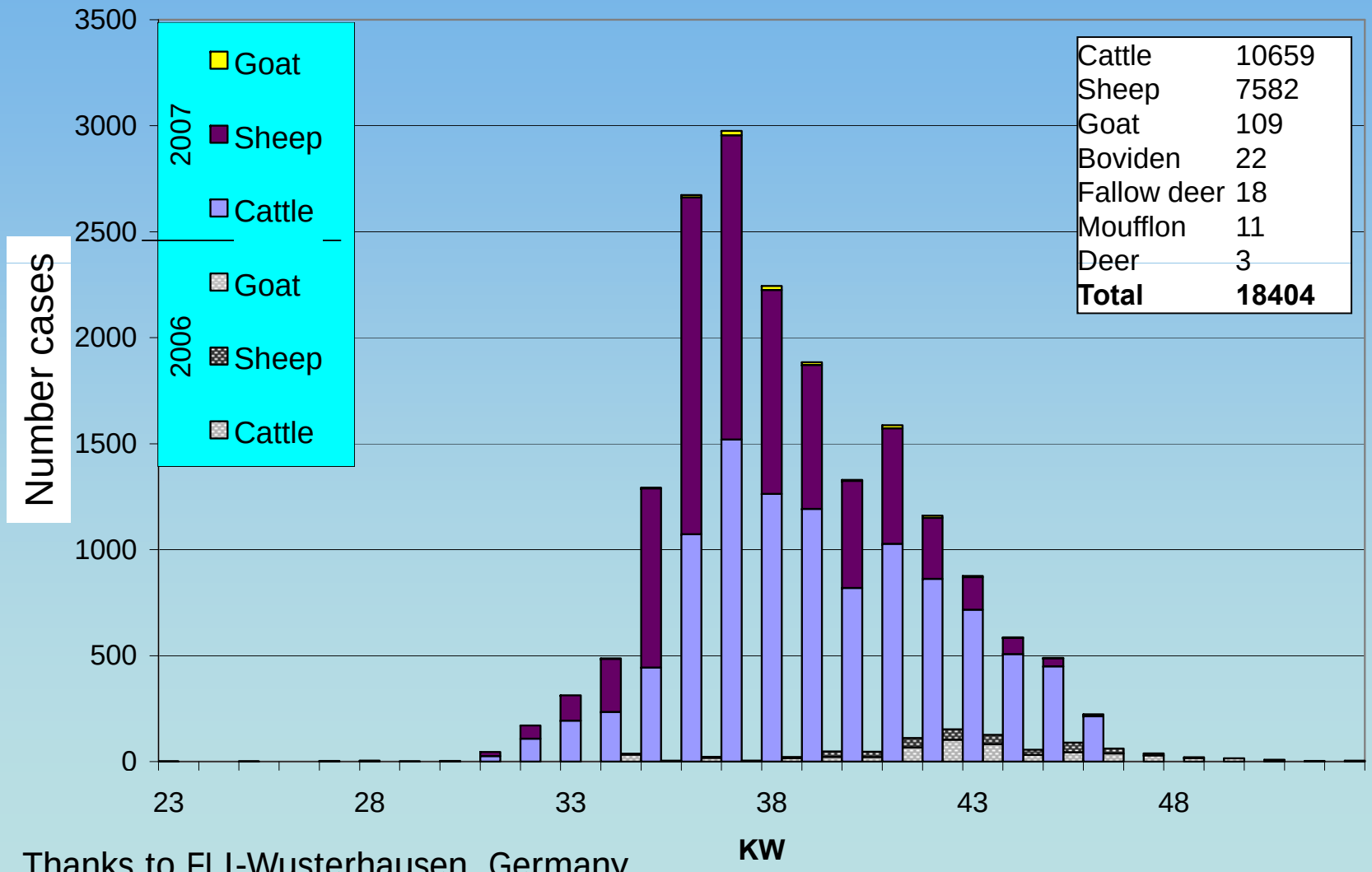
Outbreaks 2006/2007



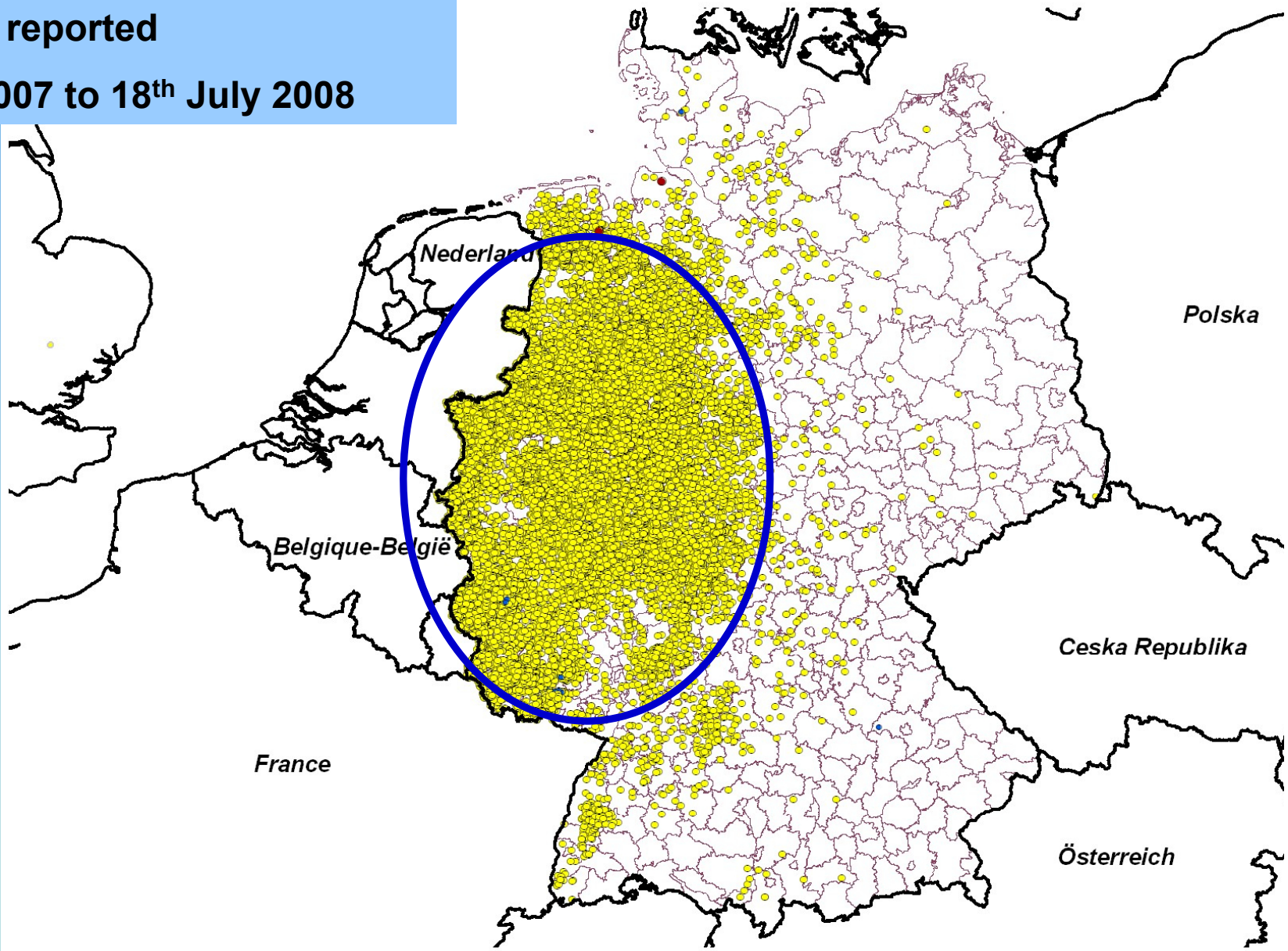
Thanks to FLI-Wusterhausen, Germany

Status: 16.11.2007

Bluetongue in Germany

2033 cases reported

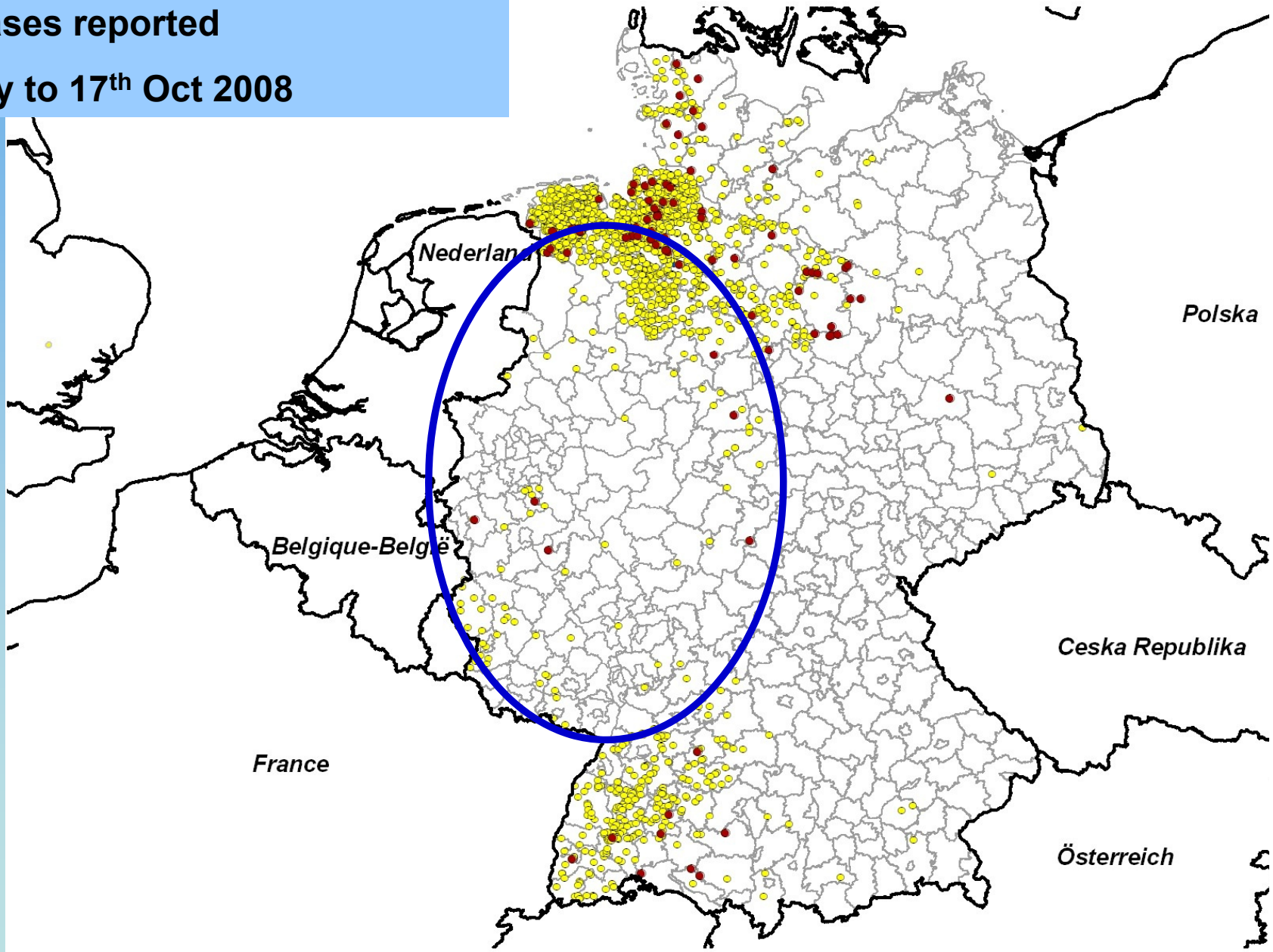
1st June 2007 to 18th July 2008

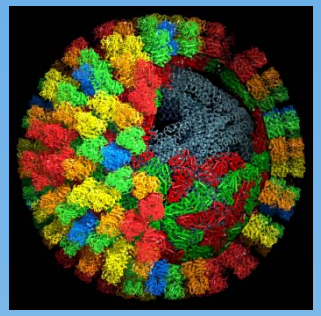


Bluetongue in Germany

2033 cases reported

1st May to 17th Oct 2008





BTV-8 in France

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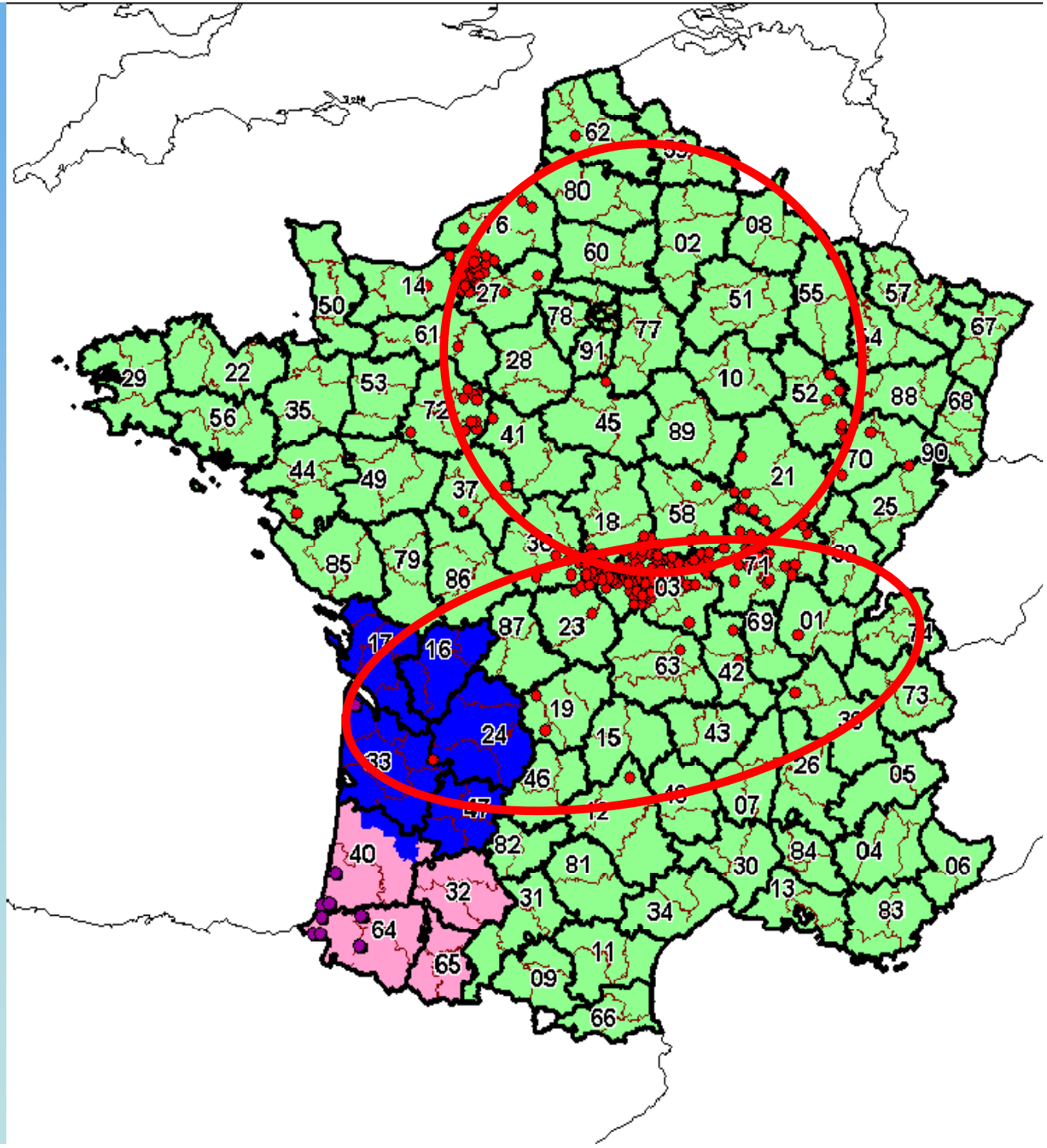
Outbreaks caused up to start of August 2008 by:
BTV-8 ●
and
BTV-1 ●



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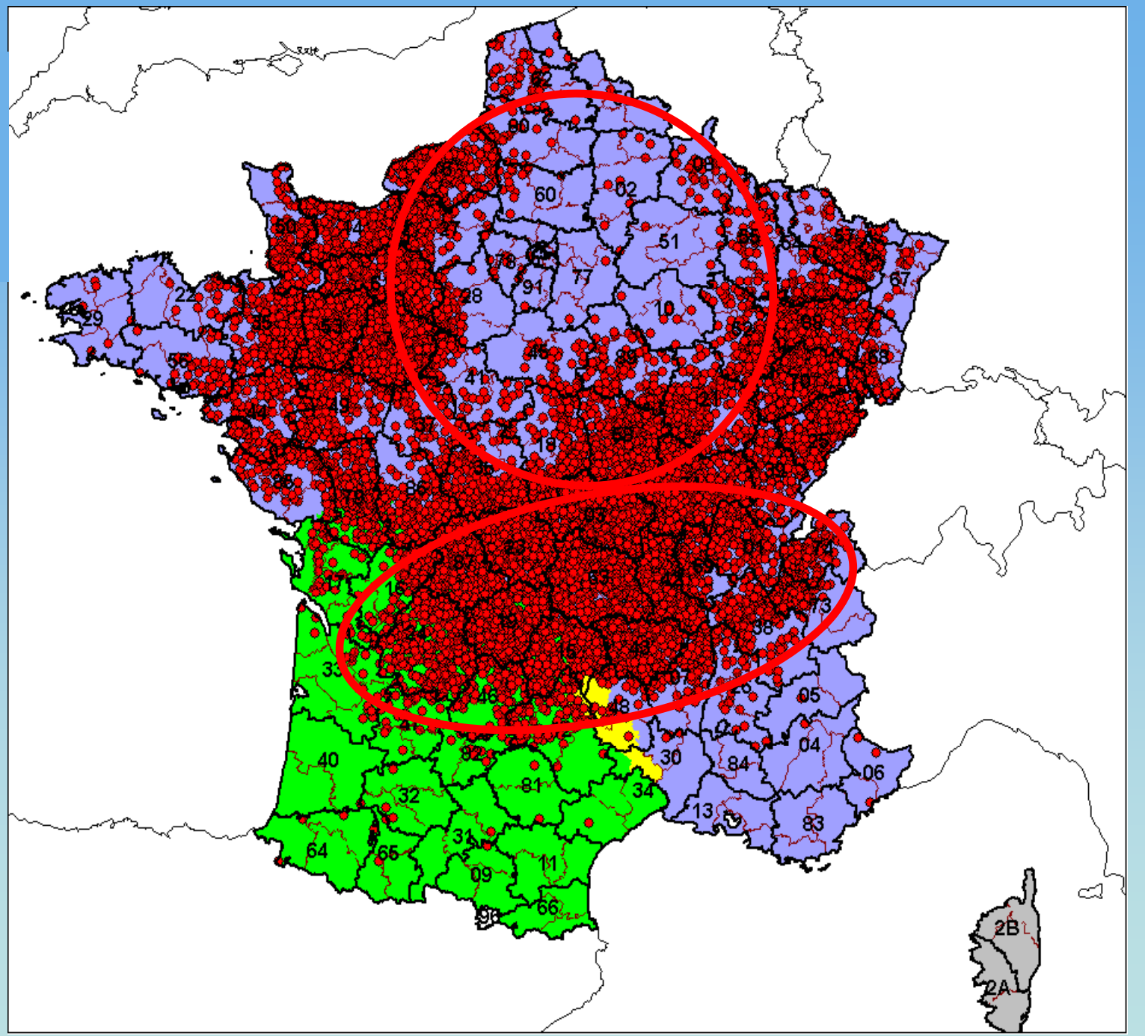
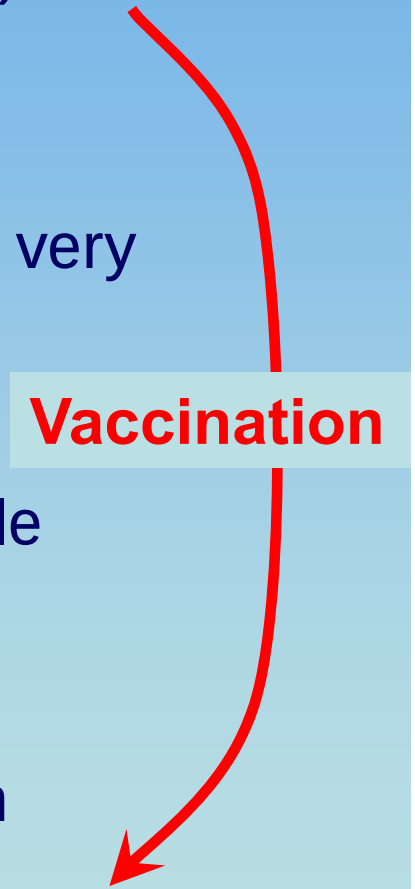
Outbreaks caused up to Late October 2008 by :
BTV-8 ●

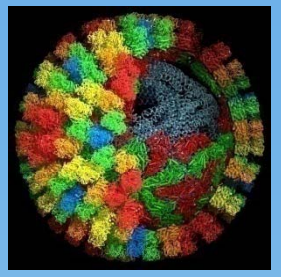


BTV-8 outbreak Northern Europe

- **year 1:**, low level initial spread (mainly in cattle), low mortality.
- **2nd year:** high level spread in cattle and sheep very severe.
- **3rd year:** Recovered animals but few susceptible animals remain, so fewer cases.
- **4th year (projected)** Few sources of infection remain and most animals immune. Potential for eradication



BTV vaccines



- 3 Options
  - Live attenuated vaccine strains
    - (unsafe in Northern European sheep – transmitted in the field)
  - Inactivated virus vaccines
    - (used in the **UK**/France/Spain 2008/2009)
  - Next generation vaccines
    - (not yet commercially available)

The effect of Vaccination with live attenuated BTV strains in UK sheep

Pyrexia and viraemia duration recorded in Dorset Poll sheep after vaccination

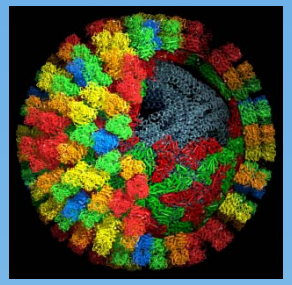
Serotype	No. of sheep showing pyrexia	Pyrexia duration range (days)	Max viraemia titre recorded*	Max viraemia duration (days)	Min viraemia duration (days)
BTV2	2/4	1	4.75	17	17
BTV4	2/4	2	4.25	16	11
BTV9	4/4	1-5	5.25	15	7
BTV16	4/4	6	6.83	19	17
BTV16	3/3	1	6.0	23	10

***(log10TCID/50)**

Veronesi E. et al., Live attenuated bluetongue vaccine viruses in Dorset Poll sheep before and after the passage in vector midges (Diptera: *Ceratopogonidae*). *Vaccine 2005; 23: 5509-5516*

Sheep inoculated with BTV-4 vaccine virus

- Acute respiratory dysfunction
- Swelling and discolouring of the lips and tongue and copious salivation
- Cyanosis of the whole oral cavity including the tongue (acute oxygen deficiency)



After compulsory vaccination, in France with inactivated vaccines, only three isolates of BTV-8 and no isolates of BTV-1 were made during 2009

BBSRC
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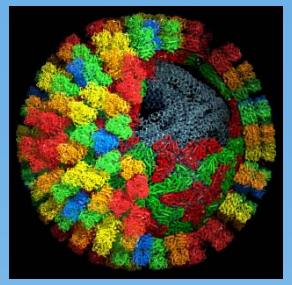

BTV in the UK during 2007-9

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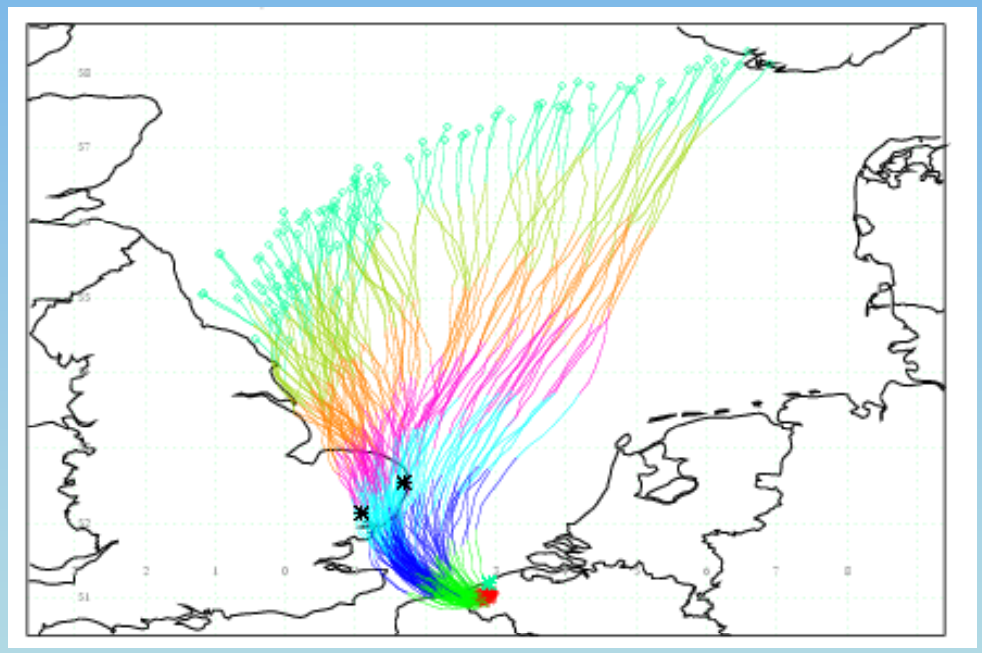
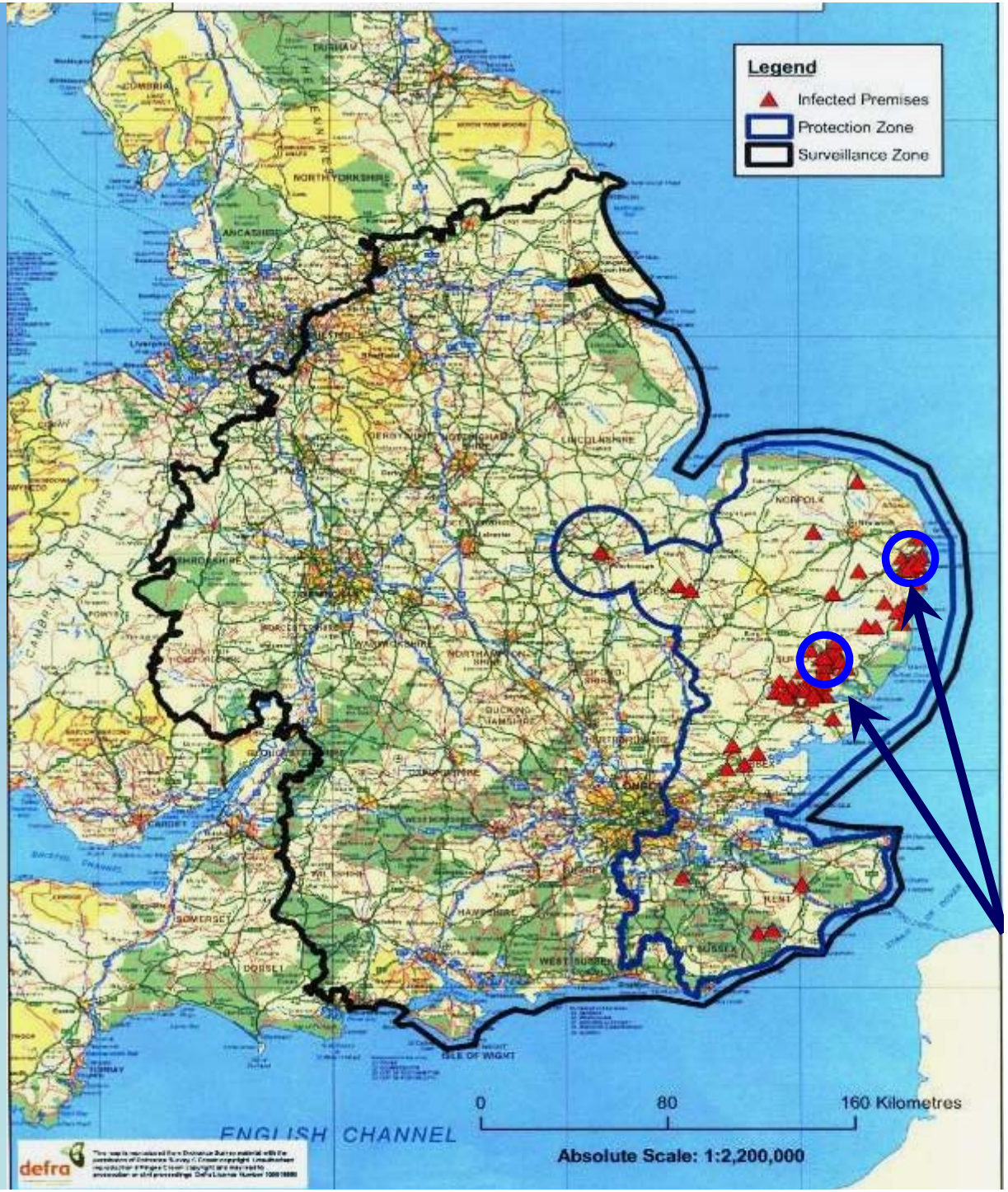
First Incursion of BTV-8 to the UK (2007)

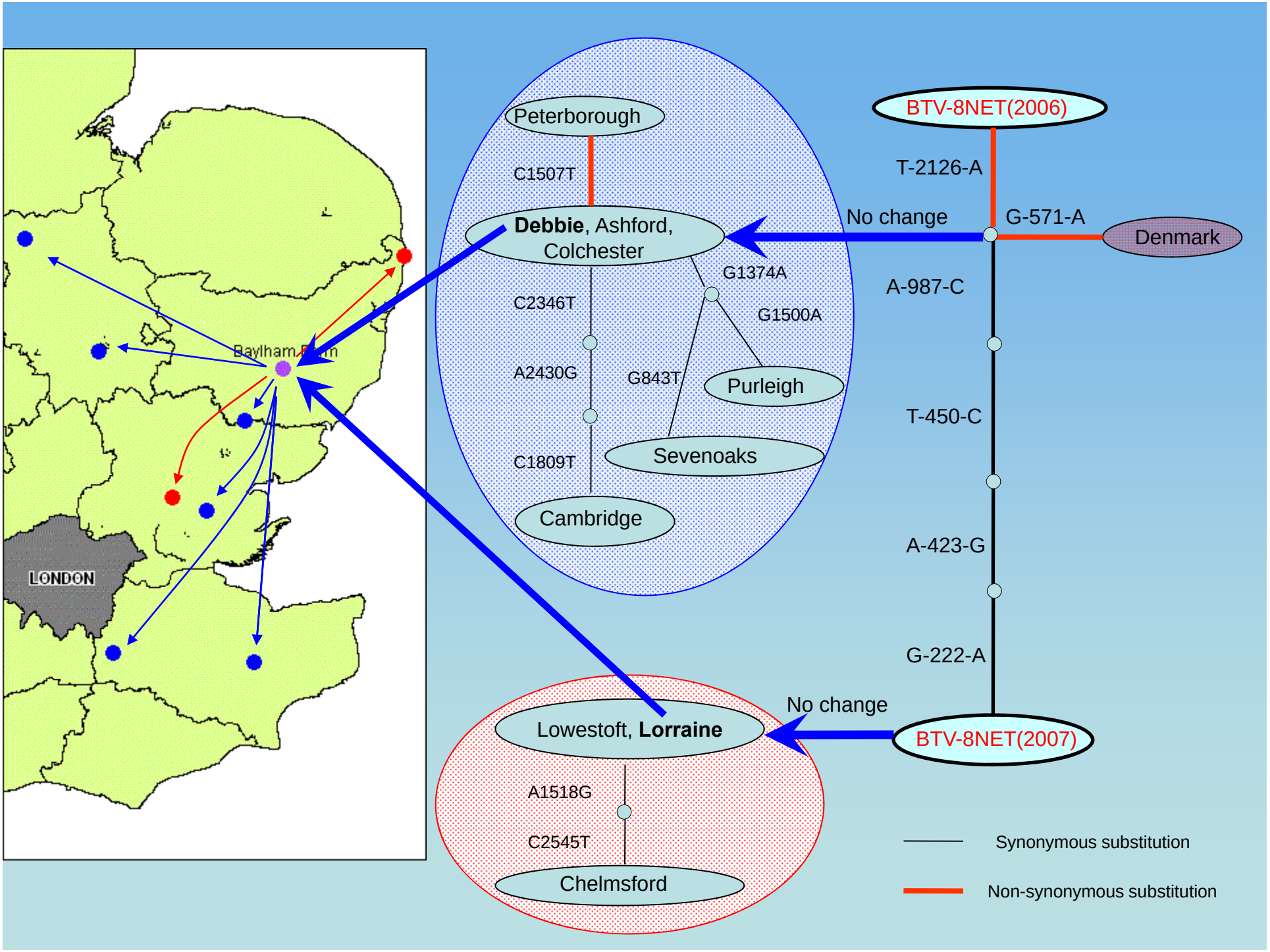


Air concentration plume and particle trajectories overnight on 4/5 Aug 2007 are believed to have caused the first outbreaks of BTV-8 in Suffolk

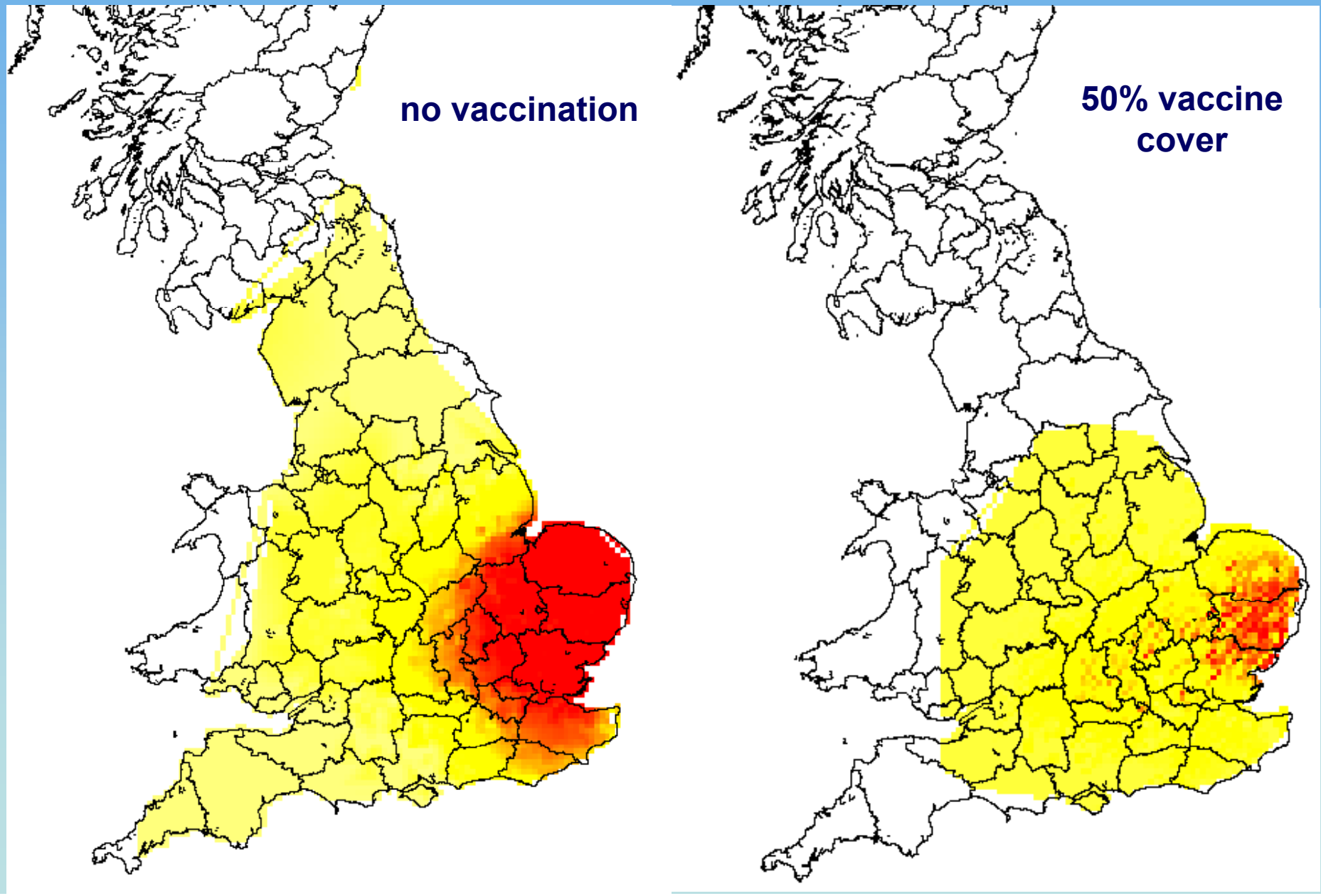
BTV-8 Protection and Surveillance zones

(1st Jan 2008)




Peterborough
C1507T
Debbie, Ashford, Colchester
C2346T
A2430G
C1809T
Cambridge
G1374A
G1500A
G843T
Purleigh
Sevenoaks
Baylham Farm
LONDON
BTV-8NET(2006)
T-2126-A
No change
G-571-A
Denmark
A-987-C
T-450-C
A-423-G
G-222-A
No change
BTV-8NET(2007)
Lowestoft, Lorraine
A1518G
C2545T
Chelmsford
Synonymous substitution
Non-synonymous substitution

October 2008: Modelling post vaccination



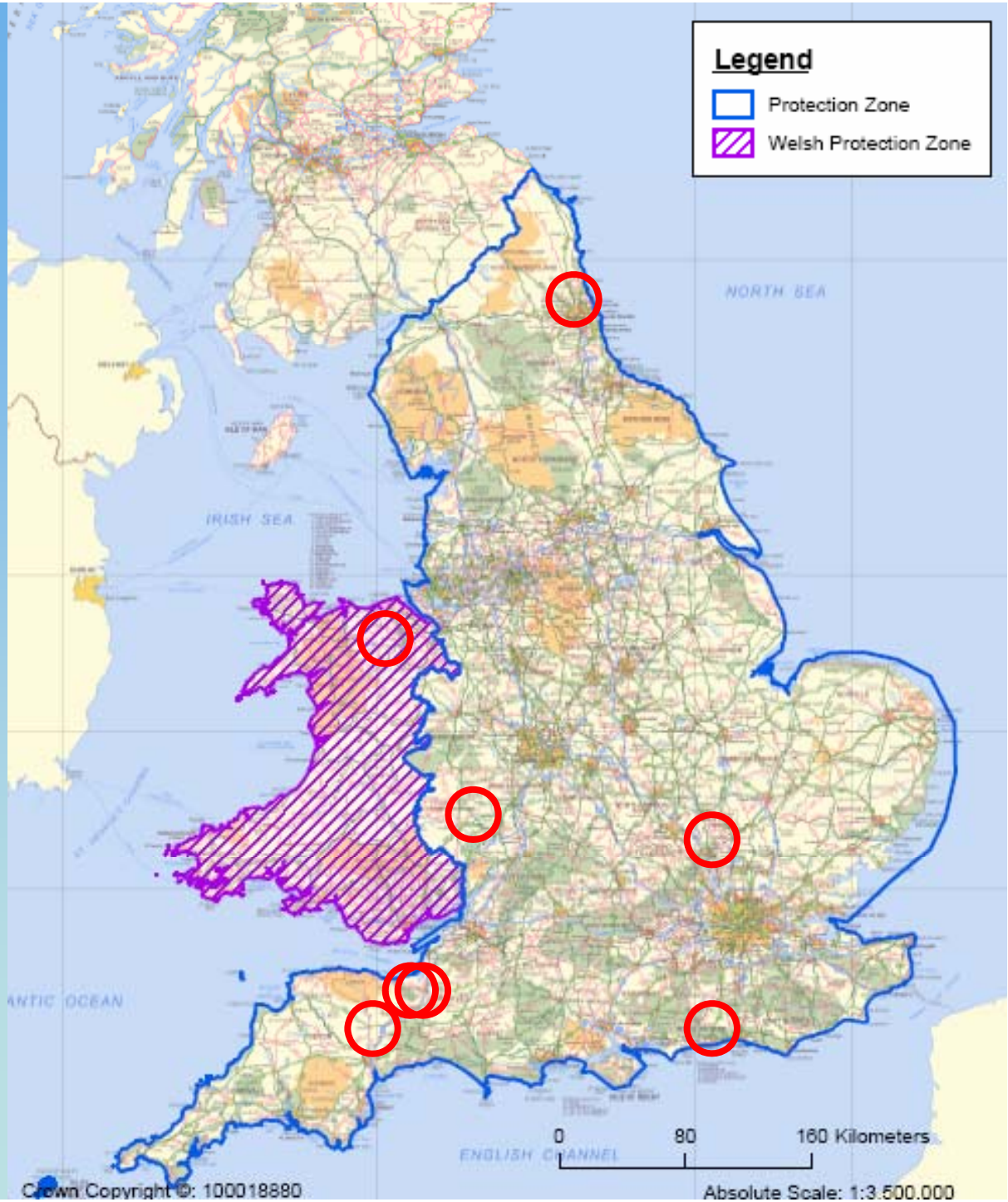
Sales of BTV-8 vaccines in the UK (to August 2008)

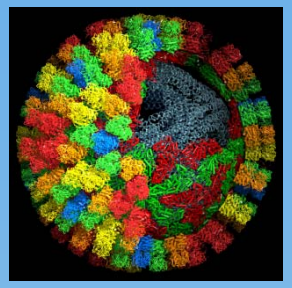
- Protection zone:
  - Total required = 11,914,834
  - Total sold = 9,853,140 **(= 82.7% cover)**

- Outside the PZ
  - Total Required = >>10,000.000
  - Total sold (so far) = 1,230,000

Zones as of Monday 20th October 2008:

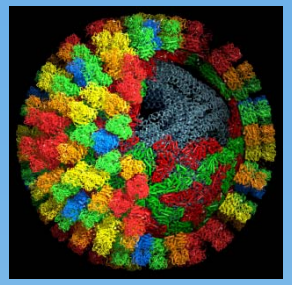
No insect transmission but importation of 196 viraemic animals





No further cases of Insect transmitted BTV were observed after the intensive vaccintion campaign against BTV-8 in the Summer of 2008

- The UK has now been reclassified a low risk zone.
- However, Vaccination in the UK has dropped to below 10%.
- It is also dropping very rapidly in the rest of northern Europe.
- As the seropositive animals are replaced, the risk of further outbreaks will inevitably return



New BTV serotypes 2008-2010

BTV-25 Switzerland

BTV-26 Kuwait

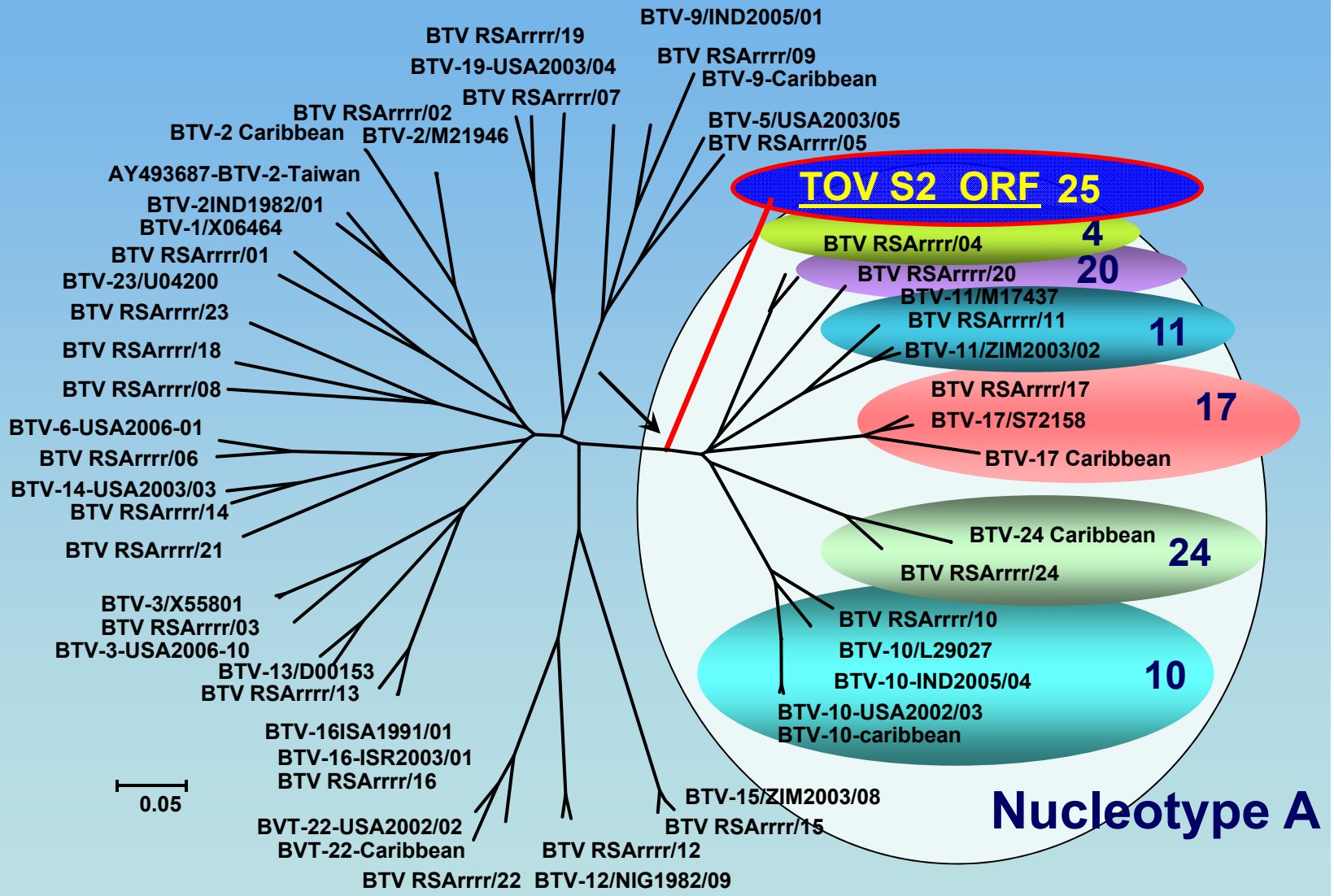
Institute for Animal Health

BTV -25 2008

Toggenburg orbivirus, Cases in goats in Switzerland

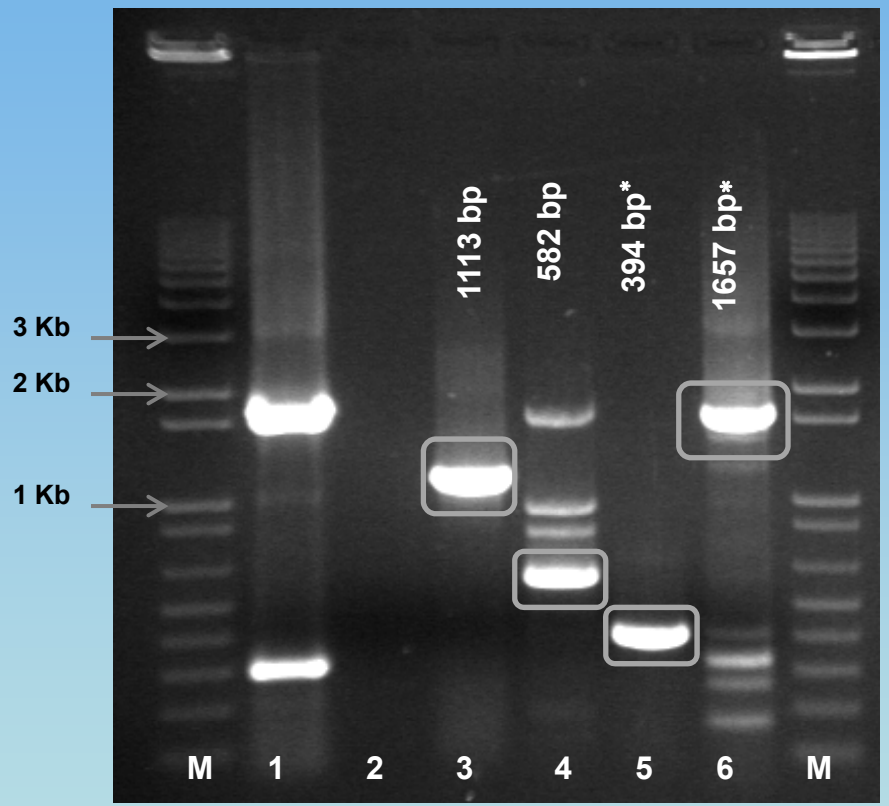
No clinical signs of infection

Phylogenetic analysis of full length Seg-2 of BTV serotypes



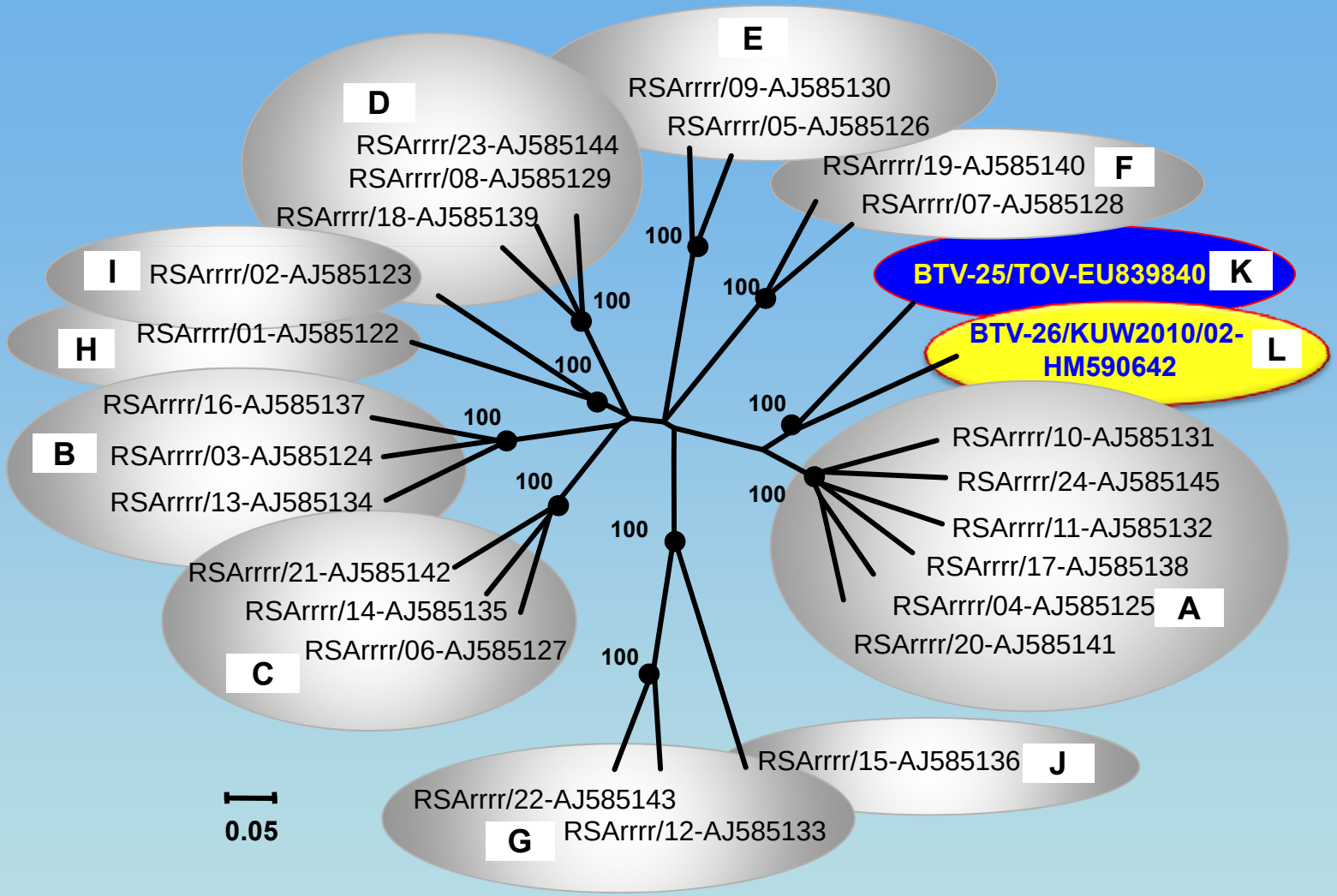
BTV -26 2010

From sheep in Kuwait 2010

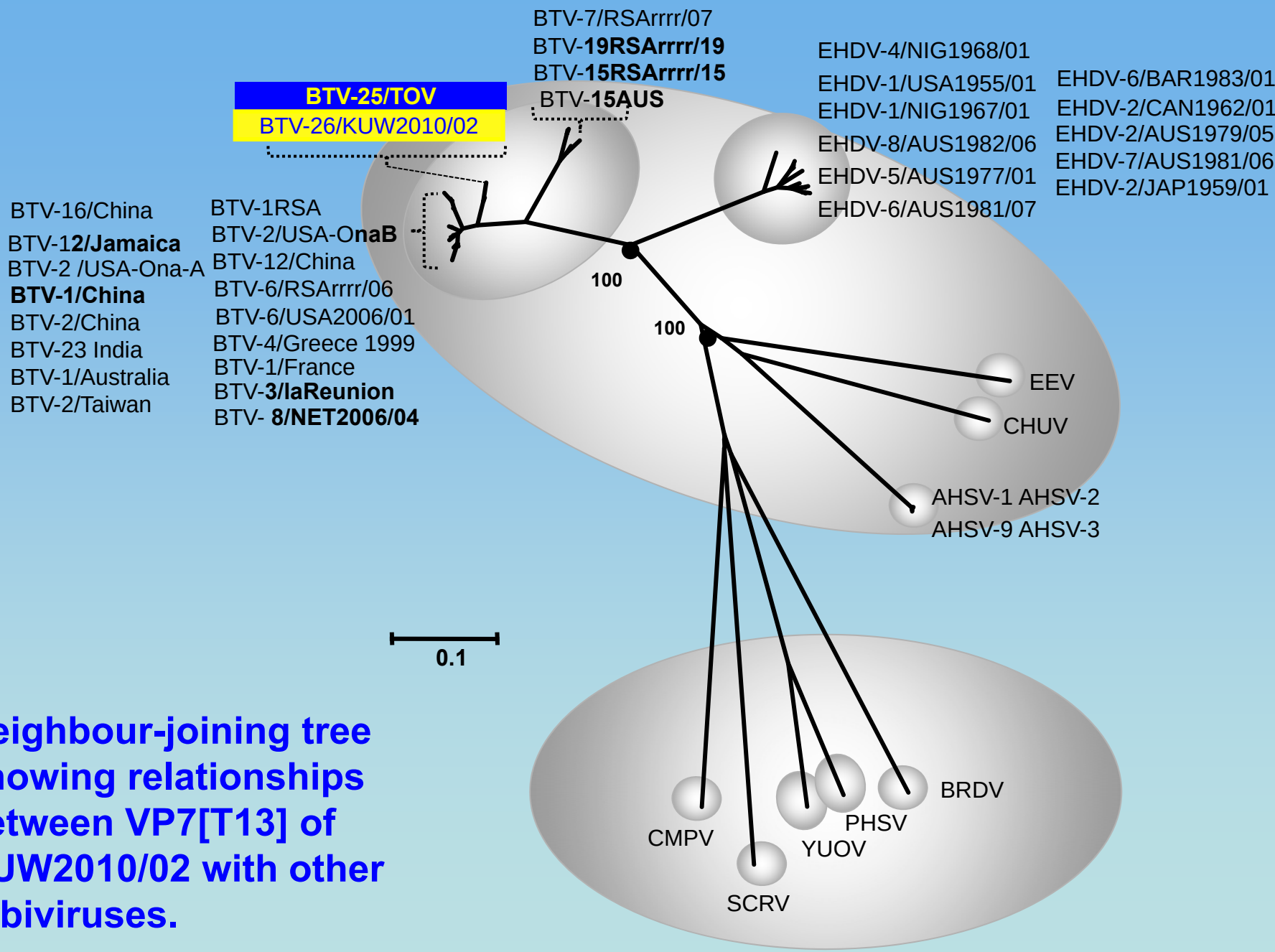


Electrophoretic analysis of cDNA products from Seg-2 of KUW2010/02 using BTV-26 specific Seg-primer-pairs.

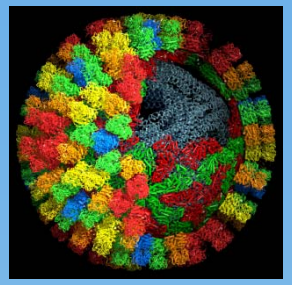
Maan et al ., Identification of a novel bluetongue virus serotype (BTV-26) from Kuwait
(Emerging Infectious Diseases – submitted 2010)



Neighbour-joining tree showing relationships between VP2 of KUW2010/02 with BTV 1-25.



Neighbour-joining tree showing relationships between VP7[T13] of KUW2010/02 with other orbiviruses.



New Virus strains and from “natural gene technology”

BTV-4

in Morocco and Spain (2010)

BTV-4 in Spain and Morocco (2010)

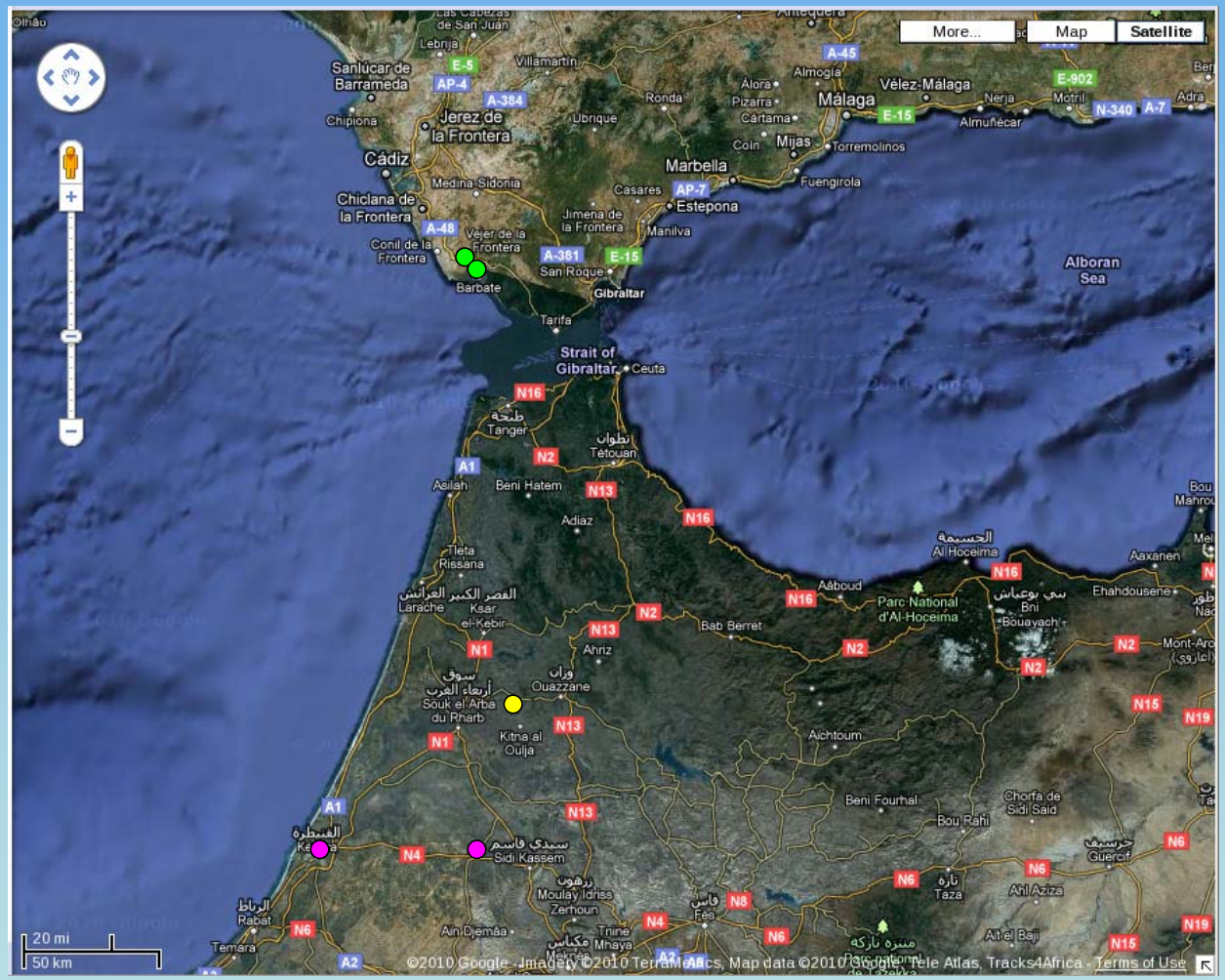
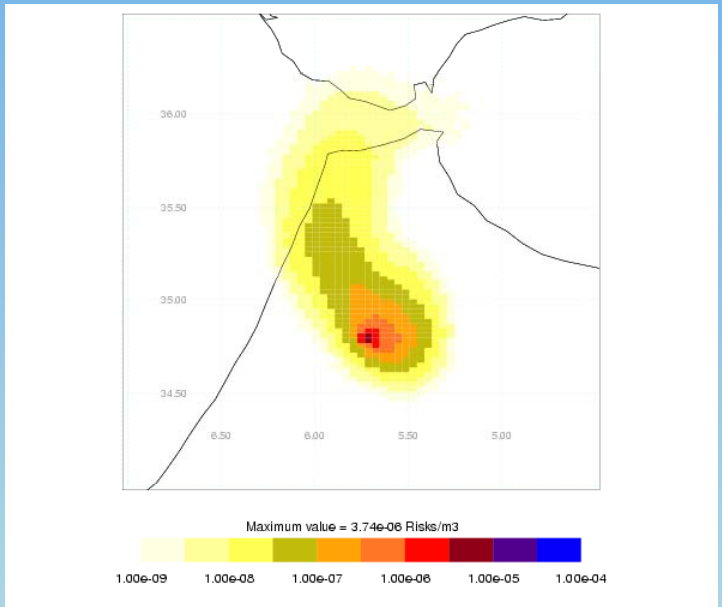
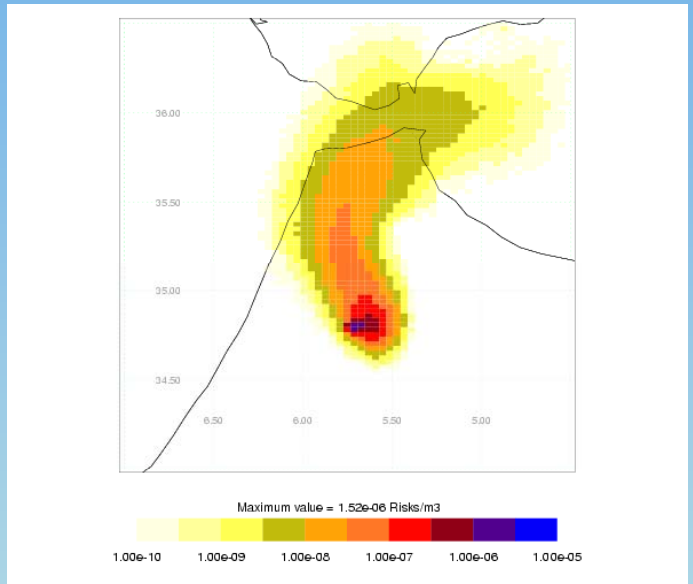


Fig 1: Locations of BTV-4 outbreaks in Spain (green dots) and Morocco (pink and yellow dots). The release location for the forward NAME runs is identified by the yellow dot.

Wind-borne spread of BTV-4 to Spain



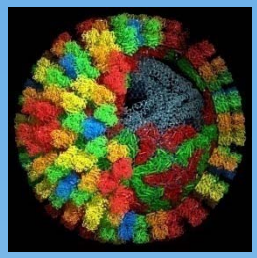


From Climate modelling (NAME) BTV-4 could have spread from Morocco into Spain on the following dates: 20-21st, 29-30th, 15-16th, or 16-17th August 2010

Similarity between BTV-4 2009 (MOR 2009/09) and previous strains of BTV 1 and 4 from Morocco

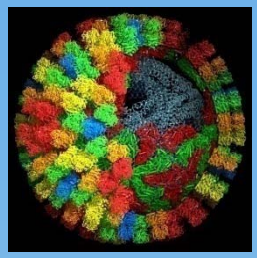
BTV-4 MOR 2004/02 % similarity with MOR 2009-09	BTV-4 Morocco 2009 (MOR 2009-09) Genome segment Number	BTV-1 MOR 2006/06 % similarity with MOR 2009-09
98.8%	1	99.8%
99.6%	2	49.9%
99.4%	3	94.90%
95.9%	4	99.8%
96.8%	5	100%
99.8%	6	69.2%
94.1% (4MOR2009/10)	7	100%
-	8	-
-	9	-
-	10	-

Summary

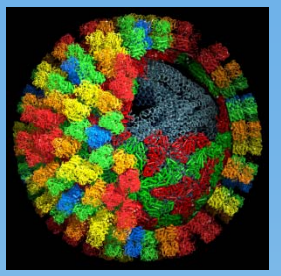


- New European BTV strains every year since 1998 . What will arrive next?
- Multiple serotypes (e.g. BTV-1, 4, 6, 8 and 11) make detection and control (vaccination) more difficult .
- The whole of Europe is now ‘at risk’ from further incursions of BTV, (new serotypes)
- Genetic exchange (reassortment) can lead to novel virus strains (which may be more dangerous)
- New viruses will emerge, even within well characterised genera and species (BTV-25 and 26).
- New cross-reactive vaccines are needed
- Europe is under threat from other orbiviruses (e.g. AHSV and EHDV), and other arboviruses Recent outbreaks of West Nile, Chicungunya,

Biosafety Considerations



- What containment level is appropriate for work on BTV
  - transfer of reagents from foreign animal disease labs to lregionaldiagnostic labs
  - transfer of exotic virus strains to low containment labs
- Vector-borne transmission is most relevant in the field but not exclusive
  - -oral transmission has been observed in the field



Acknowledgements

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