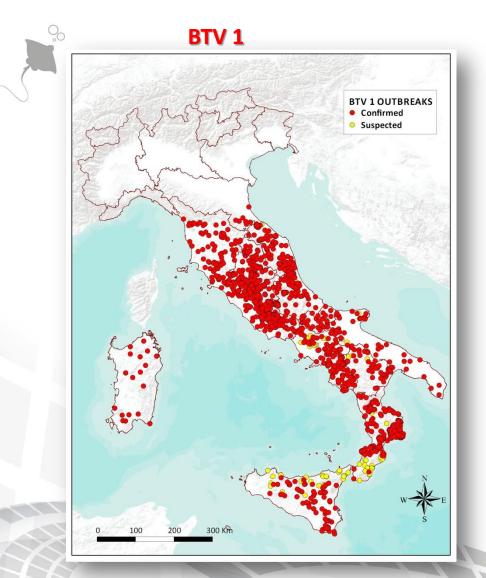


Bluetongue in Italy

22/05/2015



BT- Epidemiological situation in Italy BTV 1 and BTV 4 outbreaks – 2014



BTV 4





BT- Epidemiological situation in Italy outbreaks - 2014

| | | Cattle/buffaloes | | | | Sheep/goats | | | | | | | | | | |
|-------------------|----------|-----------------------|-------------------------------|-------------|-------|------------------------|--------|------------------|------------------|-------------------------------|-------------|-------|------------------------|--------|------------------|------------------|
| Region | Serotype | Confirmed outbreak | No. confirmed outbreaks | Susceptible | Cases | Clinically affected | Deaths | Morbidity (%) | Mortality (%) | No. confirmed outbreaks | Susceptible | Cases | Clinically affected | Deaths | Morbidity (%) | Mortality (%) |
| | | | 1 | 2 | 3 | 4 | 5 | 3/2 | 5/2 | 1 | 2 | 3 | 4 | 5 | 3/2 | 5/2 |
| ABRUZZO | BTV 1 | 148 | 49 | 2030 | 212 | 9 | 1 | 10.4% | 0.0% | 99 | 18175 | 327 | 292 | 112 | 1.8% | 0.6% |
| BASILICATA | BTV 1 | 47 | 18 | 1233 | 58 | 7 | 0 | 4.7% | 0.0% | 29 | 4053 | 127 | 100 | 59 | 3.1% | 1.5% |
| CALABRIA | BTV 1 | 359 | 77 | 3679 | 323 | 15 | 0 | 8.8% | 0.0% | 282 | 61201 | 10371 | 7210 | 2339 | 16.9% | 3.8% |
| CALADRIA | BTV 2 | 2 | 1 | 39 | 2 | 0 | 0 | 5.1% | 0.0% | 1 | 113 | 1 | 0 | 0 | 0.9% | 0.0% |
| CAMPANIA | BTV 1 | 138 | 97 | 4338 | 463 | 157 | 0 | 10.7% | 0.0% | 41 | 4824 | 136 | 102 | 11 | 2.8% | 0.2% |
| EMILIA ROMAGNA | BTV 1 | 25 | 13 | 790 | 61 | 0 | 2 | 7.7% | 0.3% | 12 | 635 | 26 | 4 | 3 | 4.1% | 0.5% |
| LAZIO | BTV 1 | 335 | 48 | 4444 | 167 | 2 | 0 | 3.8% | 0.0% | 287 | 68517 | 3614 | 3115 | 2373 | 5.3% | 3.5% |
| MARCHE | BTV 1 | 90 | 32 | 1639 | 76 | 1 | 0 | 4.6% | 0.0% | 58 | 15512 | 1320 | 708 | 793 | 8.5% | 5.1% |
| MOLISE | BTV 1 | 35 | 30 | 1792 | 100 | 0 | 0 | 5.6% | 0.0% | 5 | 733 | 26 | 14 | 22 | 3.5% | 3.0% |
| PUGLIA | BTV 1 | 63 | 56 | 1996 | 74 | 0 | 0 | 3.7% | 0.0% | 7 | 1270 | 67 | 62 | 19 | 5.3% | 1.5% |
| 1 OCLIA | BTV 4 | 38 | 32 | 973 | 43 | 0 | 0 | 4.4% | 0.0% | 6 | 1203 | 25 | 20 | 3 | 2.1% | 0.2% |
| SARDINIA | BTV 1 | 20 | 5 | 383 | 8 | 1 | 0 | 2.1% | 0.0% | 15 | 4686 | 141 | 20 | 14 | 3.0% | 0.3% |
| SICILY | BTV 1 | 82 | 71 | 5592 | 212 | 2 | 0 | 3.8% | 0.0% | 11 | 1430 | 51 | 48 | 39 | 3.6% | 2.7% |
| TUSCANY | BTV 1 | 91 | 44 | 3606 | 157 | 0 | 0 | 4.4% | 0.0% | 47 | 8981 | 261 | 242 | 57 | 2.9% | 0.6% |
| UMBRIA | BTV 1 | 131 | 14 | 896 | 57 | 0 | 0 | 6.4% | 0.0% | 117 | 18460 | 677 | 657 | 401 | 3.7% | 2.2% |
| TOTAL | | 1604 | 587 | 33430 | 2013 | 194 | 3 | 6.0% | 0.0% | 1017 | 209793 | 17170 | 12594 | 6245 | 8.2% | 3.0% |



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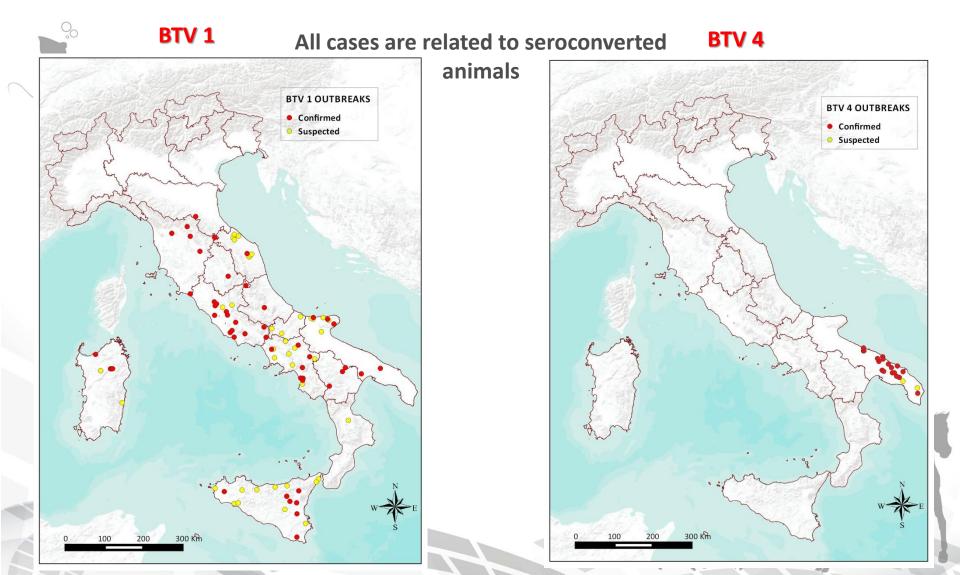
Vaccination – 2014

| Region | Serotype | Cattle and buffaloes | Sheep and goats |
|-------------|------------|----------------------|-----------------|
| ABRUZZO | BTV1 | 40 | 10 425 |
| ADRUZZU | BTV1-BTV8 | 89 | 851 |
| BASILICATA | BTV1-BTV8 | 6 499 | 17 070 |
| CALABRIA | BTV1-BTV8 | 5 681 | 334 |
| CAMPANIA | BTV1 | 22 | - |
| CAIVIPAINIA | BTV1-BTV8 | 546 | 29 |
| LAZIO | BTV1 | 377 | 757 |
| LAZIO | BTV1-BTV8 | 16 599 | 66 843 |
| LIGURIA | BTV1 | 78 | - |
| LIGORIA | BTV1-BTV8 | 1 336 | 257 |
| MARCHE | BTV1 | 47 | 3 115 |
| | BTV1 | 62 150 | 965 853 |
| SARDINIA | BTV1-BTV8 | 11 581 | 858 648 |
| SICILY | BTV1-BTV8 | 2 284 | 344 |
| THECONY | BTV1 | 276 | 5 |
| TUSCANY | BTV1-BTV8 | 2 361 | 2 512 |
| UMBRIA | BTV1 | 1 376 | 739 |
| TO | TAL | 111 342 | 1 927 782 |

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BT- Epidemiological situation in Italy BTV 1 and BTV 4 occurence 1/01/2015-22/05/2015





BT- Epidemiological situation in Italy 1/01/2015-22/05/2015

| Region | Region Serotype | | Suspected | Total |
|----------------|-----------------------|----|-----------|-------|
| ABRUZZO | BTV 1 | 1 | | 1 |
| BASILICATA | BTV 1 | 3 | | 3 |
| CALABRIA | BTV 1 | | 1 | 1 |
| CAMPANIA | BTV 1 | 8 | 7 | 15 |
| CAMPANIA | Serotyping is ongoing | | 2 | 2 |
| EMILIA ROMAGNA | BTV 1 | 1 | | 1 |
| LAZIO | BTV 1 | 14 | 2 | 16 |
| MARCHE | BTV 1 | 1 | 4 | 5 |
| MARCHE | Serotyping is ongoing | | 4 | 4 |
| MOLISE | BTV 1 | | 3 | 3 |
| | BTV 1 | 5 | 4 | 9 |
| PUGLIA | BTV 4 | 18 | 2 | 20 |
| | Serotyping is ongoing | | 1 | 1 |
| | BTV 1 | 4 | 1 | 5 |
| SARDINIA | Serotyping is ongoing | | 2 | 2 |
| | BTV 1 | 8 | 11 | 19 |
| SICILY | BTV 2 | | 2 | 2 |
| TUSCANY | BTV 1 | 5 | | 5 |
| UMBRIA | BTV 1 | 1 | | 1 |
| TOTAL | | 69 | 46 | 115 |



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Vaccination 1/01/2015 - 22/05/2015

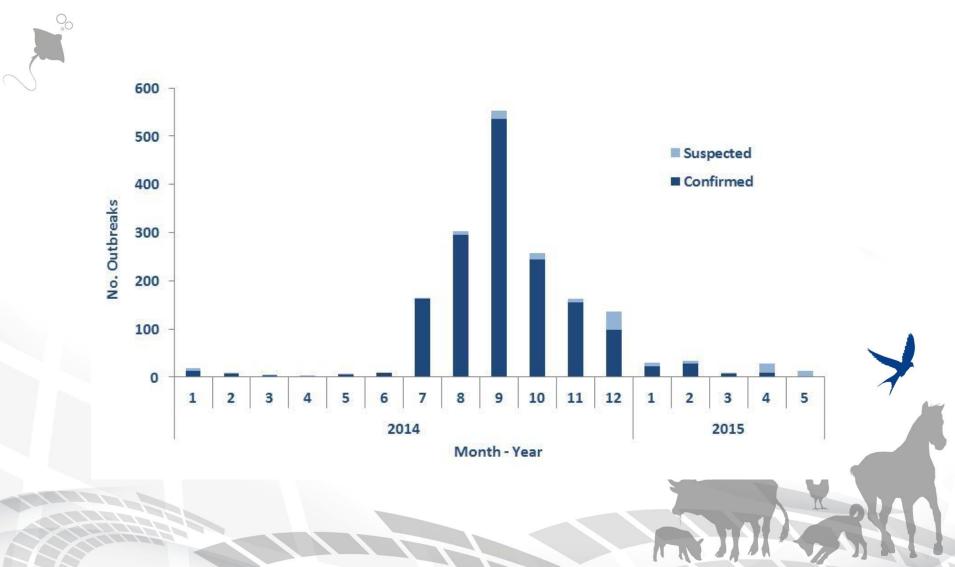
| Region | Serotype | Cattle and buffaloes | Sheep and goats |
|----------------|-----------|----------------------|-----------------|
| ABRUZZO | BTV1 | 25 | 20 304 |
| BASILICATA | BTV1 | 31 | 1 032 |
| CAMPANIA | BTV1-BTV8 | 6 | - |
| EMILIA ROMAGNA | BTV1 | 4 889 | 24 872 |
| LAZIO | BTV1 | 47 | - |
| LIGURIA | BTV1 | 283 | 205 |
| MARCHE | BTV1 | 18 359 | 59 405 |
| PUGLIA | BTV2-BTV4 | 1 089 | - |
| PUGLIA | BTV1 | 1 089 | - |
| SARDINIA | BTV1 | 14 635 | 487 993 |
| JARDINIA | BTV1-BTV8 | 3 641 | 471 364 |
| SICILY | BTV2-BTV4 | 454 | 92 |
| SICILY | BTV1 | 614 | 97 |
| TUSCANY | BTV1 | 203 | 7 |
| UMBRIA | BTV1 | 36 320 | 86 048 |
| TOTAL | | 81 685 | 1 151 419 |



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Temporal distribution of BT outbreaks in 2014 and 2015





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BTV Surveillance Programme





Bluetongue Surveillance Programme in Italy



Surveillance on sentinel animals

Large number of sentinel animals monthly tested across the whole country



Entomological surveillance

A net of *Culicoides* black-light traps weekly operated all ver the year



Bluetongue: Surveillance on sentinel animals

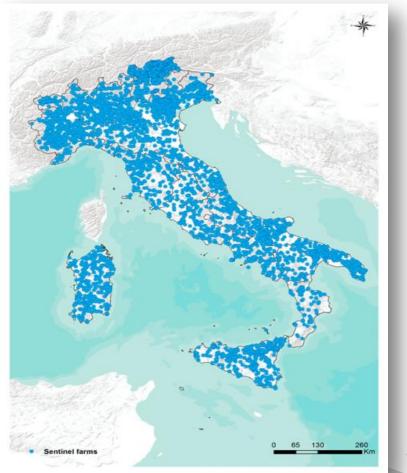


- In place since 2002
- The Italian territory has been divided in 20 x 20 km square grids
- In each square, around 58 animals are selected and used as sentinel animals

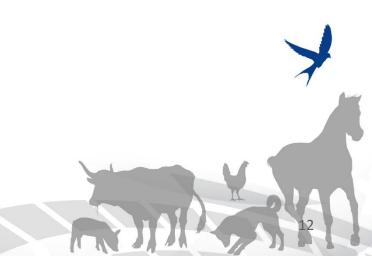


Surveillance on sentinel animals

Geographical distribution of sentinel holdings



It is a surveillance system which includes more than **30,000 animals**



Bluetongue:



Bluetongue: Surveillance on sentinel animals

They are **monthly bled** and tested by **c-ELISA**. Positive samples are confirmed by **virus neutralization assay**^(*). From positive animals EDTA blood samples are also tested by **RT-PCR**.

An automatic procedure scans these results to identify **potential seroconversions.** Laboratory tests performed on sentinel animals (cattle, sheep and goats) in Italy

| Year | No. of tested animals | No. of ELISA tests | No. of virus neutralization tests | No. of RT-PCR |
|------|--------------------------|-----------------------|---|---------------|
| 2009 | 62 705 | 476 327 | 105 472 | 6 577 |
| 2010 | 53 915 | 397 347 | 30 734 | 1 664 |
| 2011 | 51 068 | 361 736 | 20 952 | 1 012 |
| 2012 | 39 677 | 266 959 | 11 277 | 1 156 |
| 2013 | 35 657 | 255 436 | 25 585 | 1 304 |
| 2014 | 31 352 | 243 519 | 63 819 | 3 321 |

(*) For each sample tested by VN, the presence of antibodies against nine serotypes is checked: BTV-1, BTV-2, BTV-4, BTV-6, BTV-8, BTV-9, BTV-14, BTV-15, BTV-16



Bluetongue: Surveillance on sentinel animals

In all these years the sentinel system has been a **very effective and sensitive tool**, crucial for detecting BTV circulation in Italy

In 2014 around **33%** of BT outbreaks were revealed due to the sentinel animals



| Year | No. <i>Culicoides</i> collections |
|------|--------------------------------------|
| 2009 | 7 834 |
| 2010 | 9 059 |
| 2011 | 9 799 |
| 2012 | 6 806 |
| 2013 | 6 735 |
| 2014 | 8 521 |

The entomological surveillance system is used for defining seasonally free areas and to monitor vector dynamics





Bluetongue: the Italian restricted zones



An accurate and comprehensive analysis of the Italian BT situation

of the last two years was carried out to verify the possible compliance with the provisions of Commission Regulation (EC) No 1266/2007 for the Italian **restricted zones** and, where possible, to **update the actual map** of circulating BTV serotypes



Bluetongue: the Italian restricted zones

BTV-1 and BTV-4 were the mainly circulating serotypes.

Some seroconverted animals were **erroneously confirmed** for other serotypes, although these positive results were elicited by **cross reaction phenomena.**

For these cases (35 animals in 2013 and 5 in 2014) a correction procedure has been requested to ADNS.



Bluetongue: the Italian restricted zones BTV16



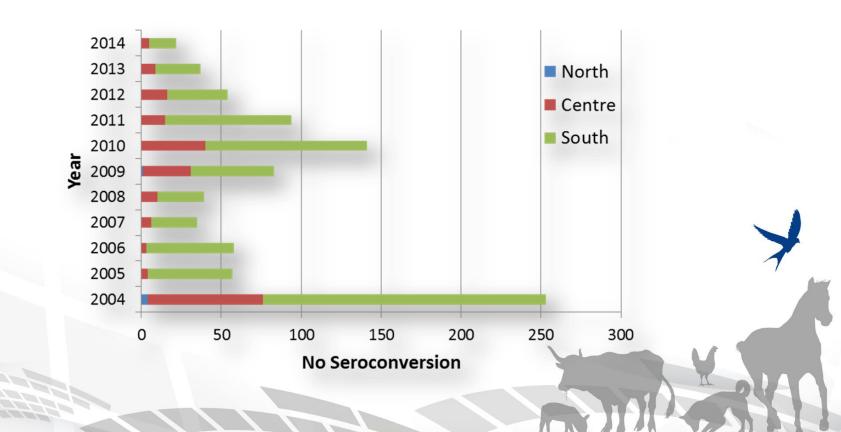
- In 2004 millions of animals were vaccinated against serotype 16 with a modified live vaccine (MLV-16) in Sardinia and in southern regions
- The MLV-16 was responsible for clinical disease in vaccinated animals, and was suspended shortly afterwards
- It has been demonstrated that the BTV-16 strain used in the vaccine was inadequately attenuated



Bluetongue: the Italian restricted zones BTV16

Number of sentinel animals which have seroconverted to BTV-16 in each Italian macro-region (NUTS1) from 2004 to 2014.

After 2004, when vaccination against BTV-16 was discontinued, clinical signs were never observed.





Bluetongue: the Italian restricted zones BTV16

- Different strains isolated from 2004 to 2007 have been
 sequenced for the whole genome by next generation sequencing
 - strain isolated from BT symptomatic sheep after vaccination with BTV-16 MLV in Sardinia in 2004
 - strain isolated from viraemic animals with no story of previous vaccination in 2006 and 2007
- All Italian BTV-16 strains were nearly identical, showing nucleotide identity 99-100%
- All gene segments of the Italian BTV-16 strains from 2004, 2006 and 2007 also showed >99% sequence identity to the reference BTV-16 MLV (RSAvvvv/16) strain

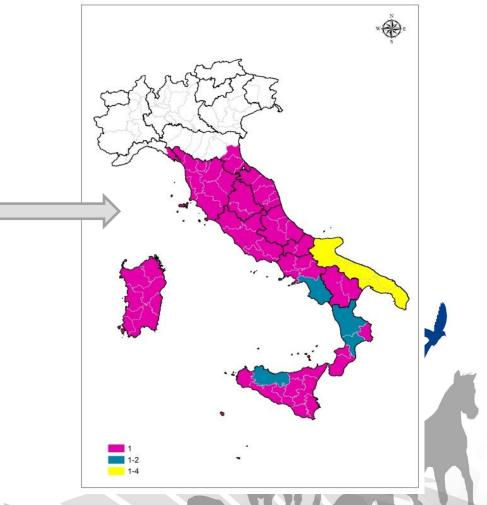


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Current restricted zones



Updated restricted zones On the basis of data analysis





Conclusions



The Italian veterinary Authority **ASKS** the competent services of the European Commission:

to reconsider the current Italian zones under restriction for BTV-16, similarly to what applied for the Netherlands and Belgium in the cases of BTV-6 and BTV-11, taking into account the vaccine origin of the BTV-16 strain.

