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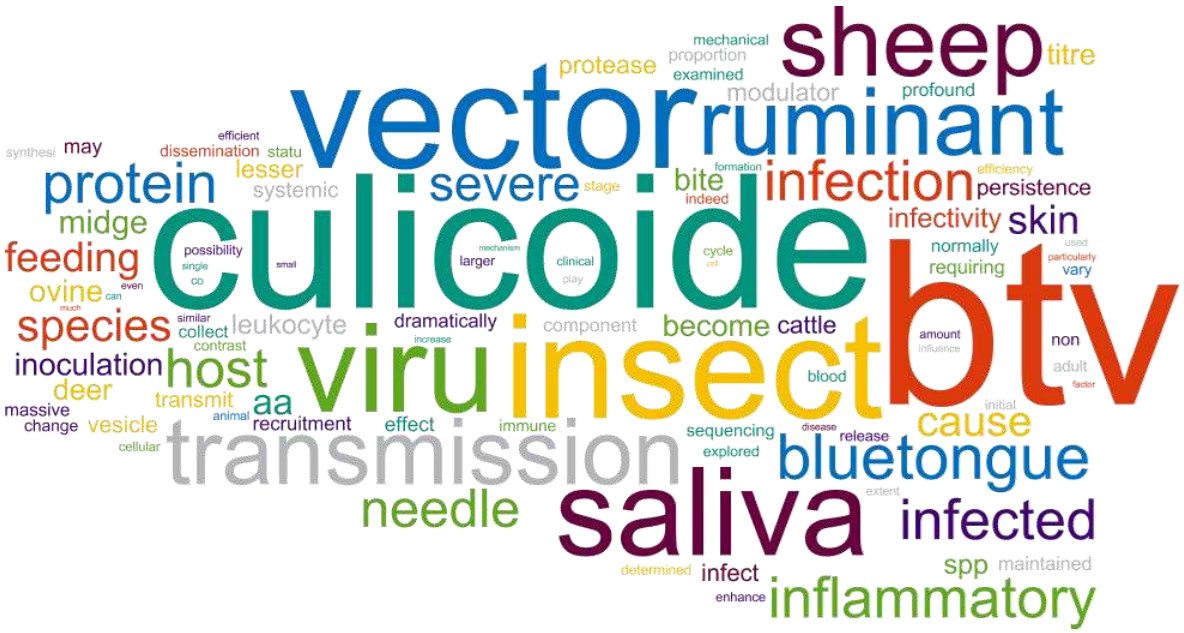
IZSLT

Focus sulla conoscenza



Il nuovo approccio alla bluetongue

Dr Andrea Carvelli

Osservatorio Epidemiologico IZSLT

Roma, 25 marzo 2021

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Definizione

- Febbre catarrale degli ovini o bluetongue
- Malattia notificabile OIE UE
- Malattia infettiva non contagiosa, trasmessa da insetti
- Ruminanti domestici e selvatici
- Sintomi da asintomatica a morte
  Variabilità: specie, razza, sierotipo, fase produttiva
- Ruolo epidemiologico dei bovini / malattia clinica ovini

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Importanza socio-economica

- Grandi perdite economiche

- + Dirette: + malattia clinica (calo produzioni)
- +++ Indirette: restrizioni alle movimentazioni di animali

- In ‘70, ban export bovine semen USA: 24.000.000 USD/year
- 2007, BTV-8 : 1,4 billions USD in France
- 2007, BTV-8: 845 millions € in Ned

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Cenni storici

1881 Hutcheon first description SA Merinos

1906 Theiler hypotized viral etiology

1908 first L-A vaccines by Theiler (BTV4)

1924 (o 43) 1st description out of Africa: Cyprus

1943 Du Toit confirmed role *Culicoides*

1956 1st Europe: Portugal and Spain (BTV10)

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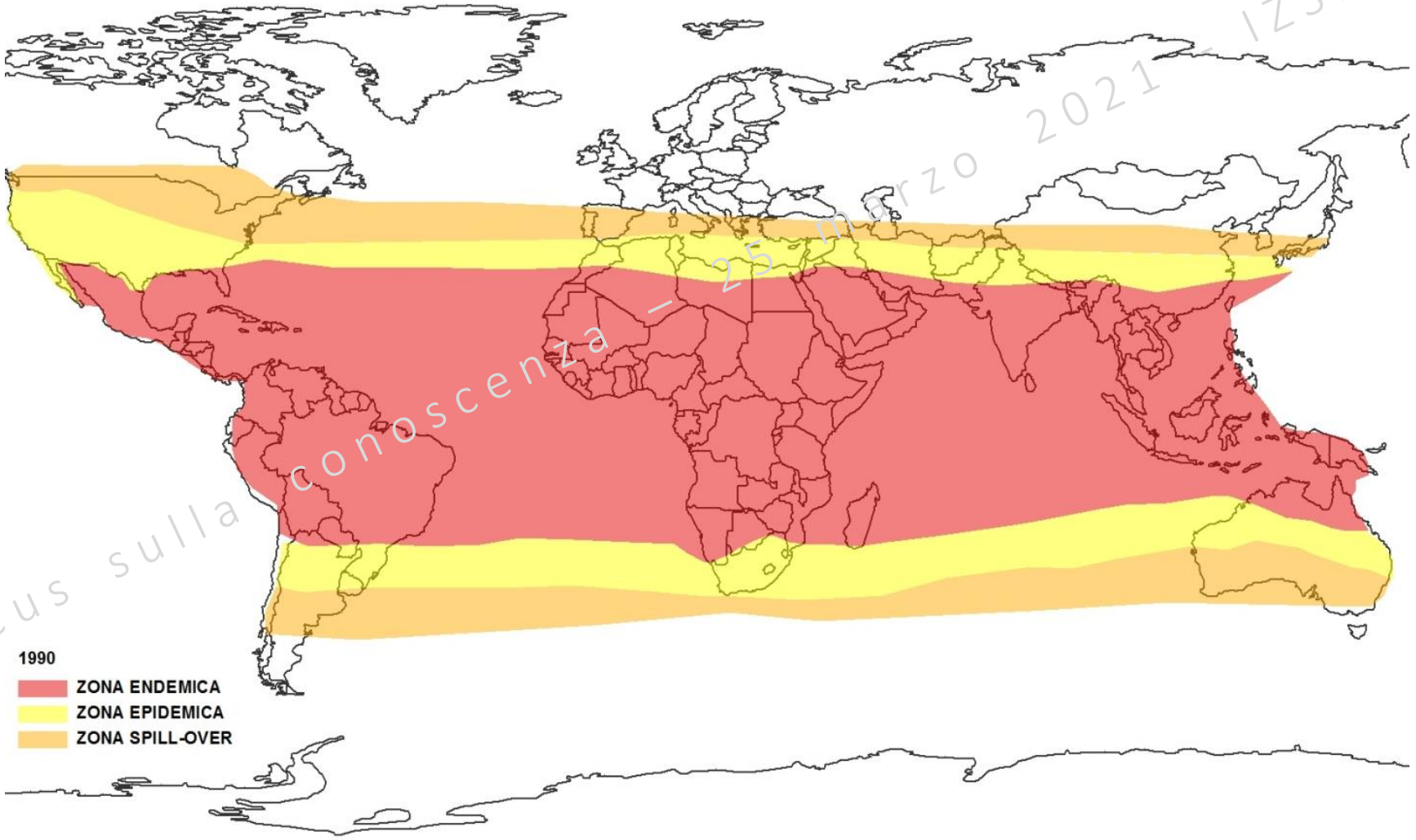
BLUETONGUE DISTRIBUTION 1990 vs 2021



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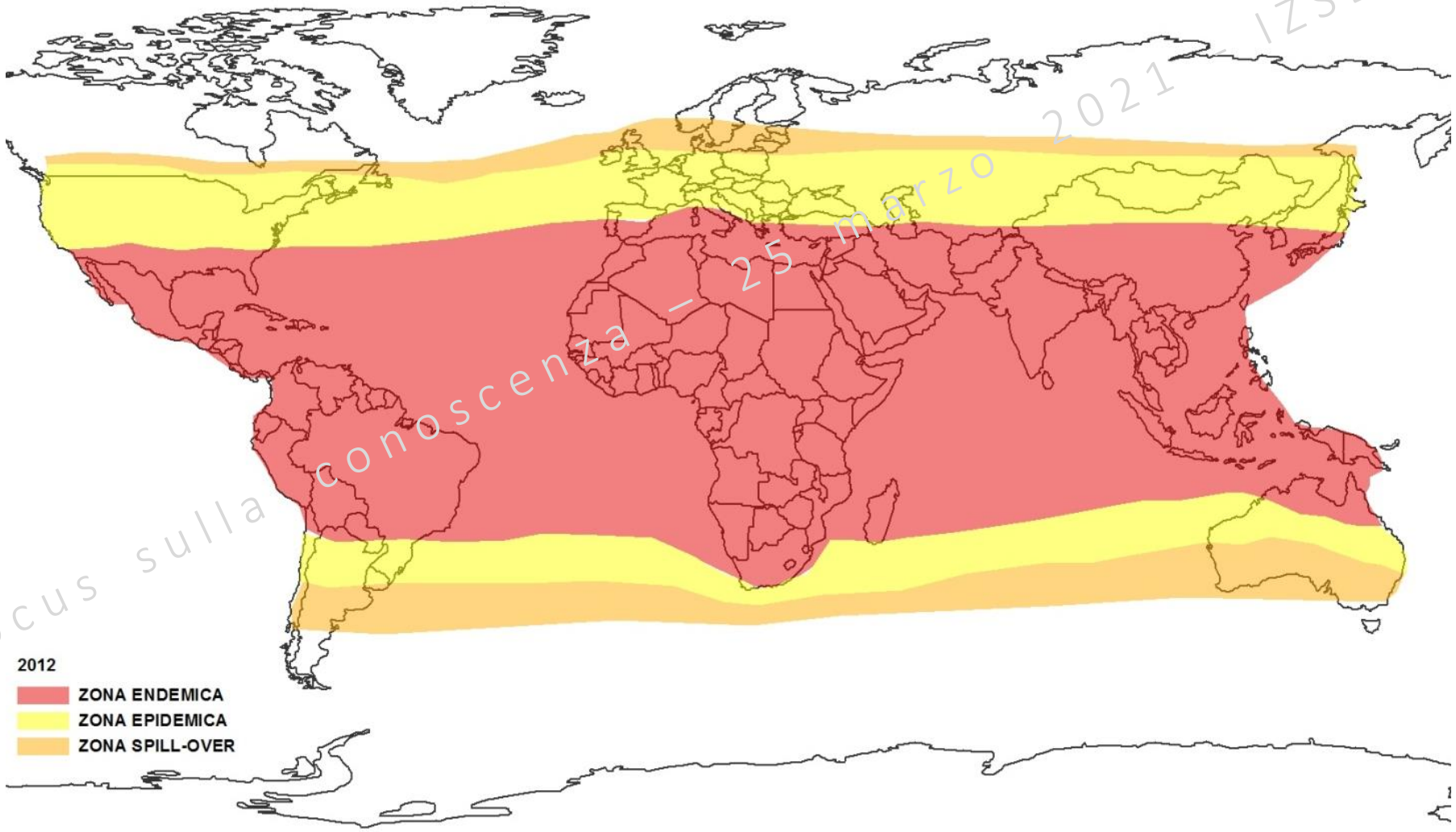
BLUETONGUE DISTRIBUTION 1990 vs 2021



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BTV OCCURRENCE 1943-2017

Source: OIE-BT-Labnet

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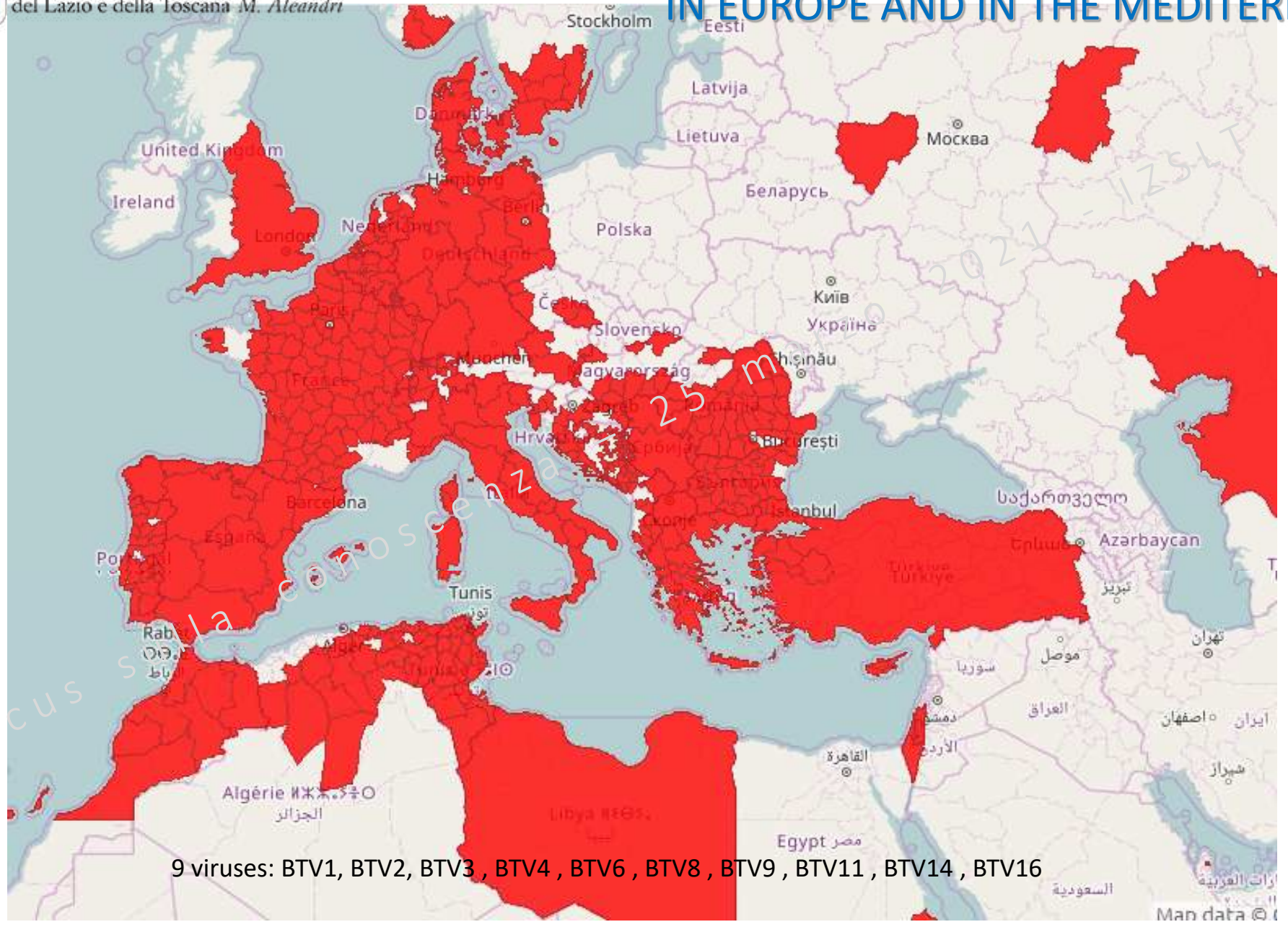
OVERALL BTV OCCURRENCE IN EUROPE AND IN THE MEDITERRANEAN BASIN 2000-2017



9 viruses: BTV1, BTV2, BTV3 , BTV4 , BTV6 , BTV8 , BTV9 , BTV11 , BTV14 , BTV16

Source: OIE-BT-Labnet

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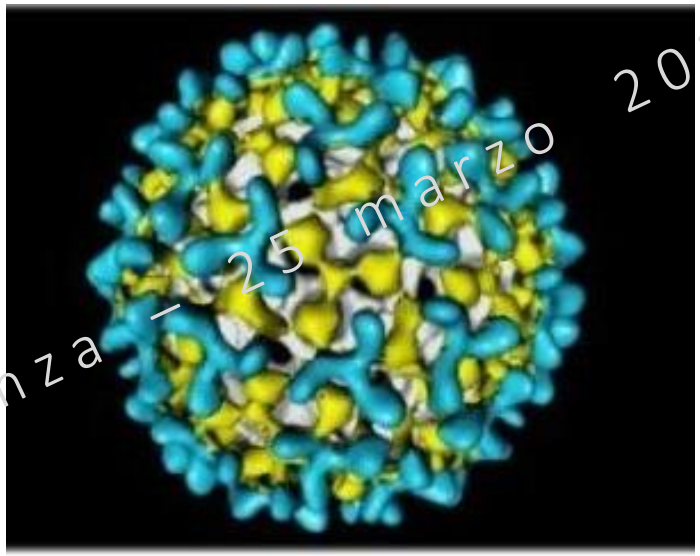
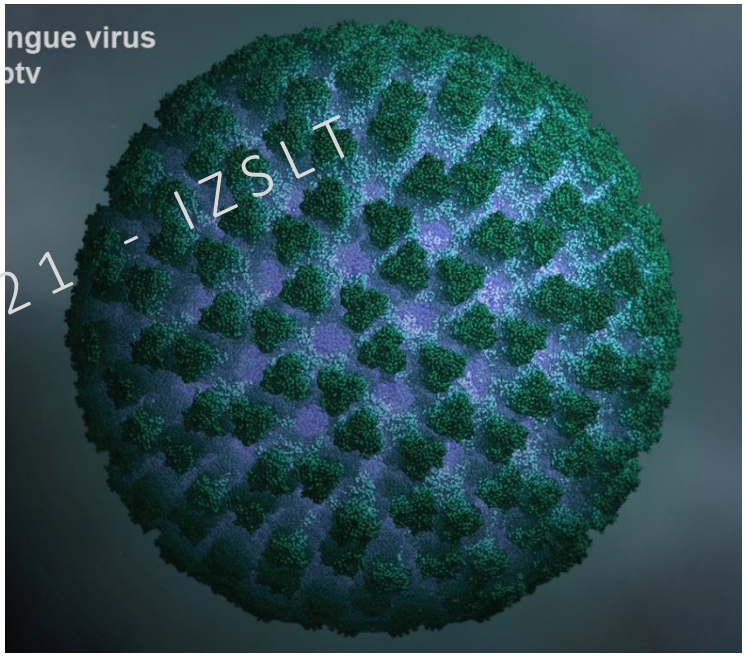
Eziologia

Family: Reoviridae

Genus: Orbivirus (ex arbo)

Serogroup: BT, AHS, EHD, EE





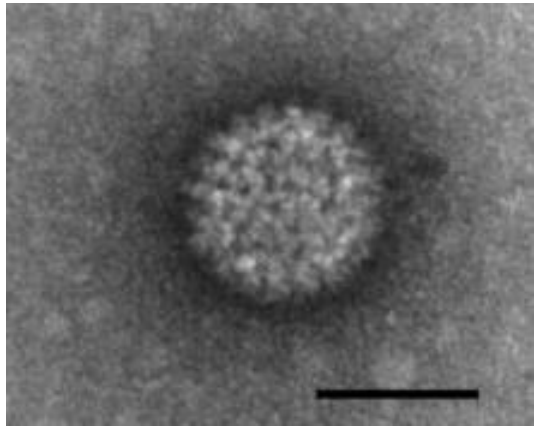


Photo: IZSAM
University
Wisconsin

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Eziologia

non-enveloped
double-stranded RNA virus
10 segments of RNA
Encodes 11 proteins 4 NS and 7 VP

Inactivated by:

- <pH 3 e >pH 8
- β-propiolactone
- Iodophors and other phenolic compound
- **labile at –20°C**
- inactivated at 50°C 3 hrs and 15 min at 60° C

Stable at

- room temperature (for 25 years)
- stable when with proteins (blood at 20° C)

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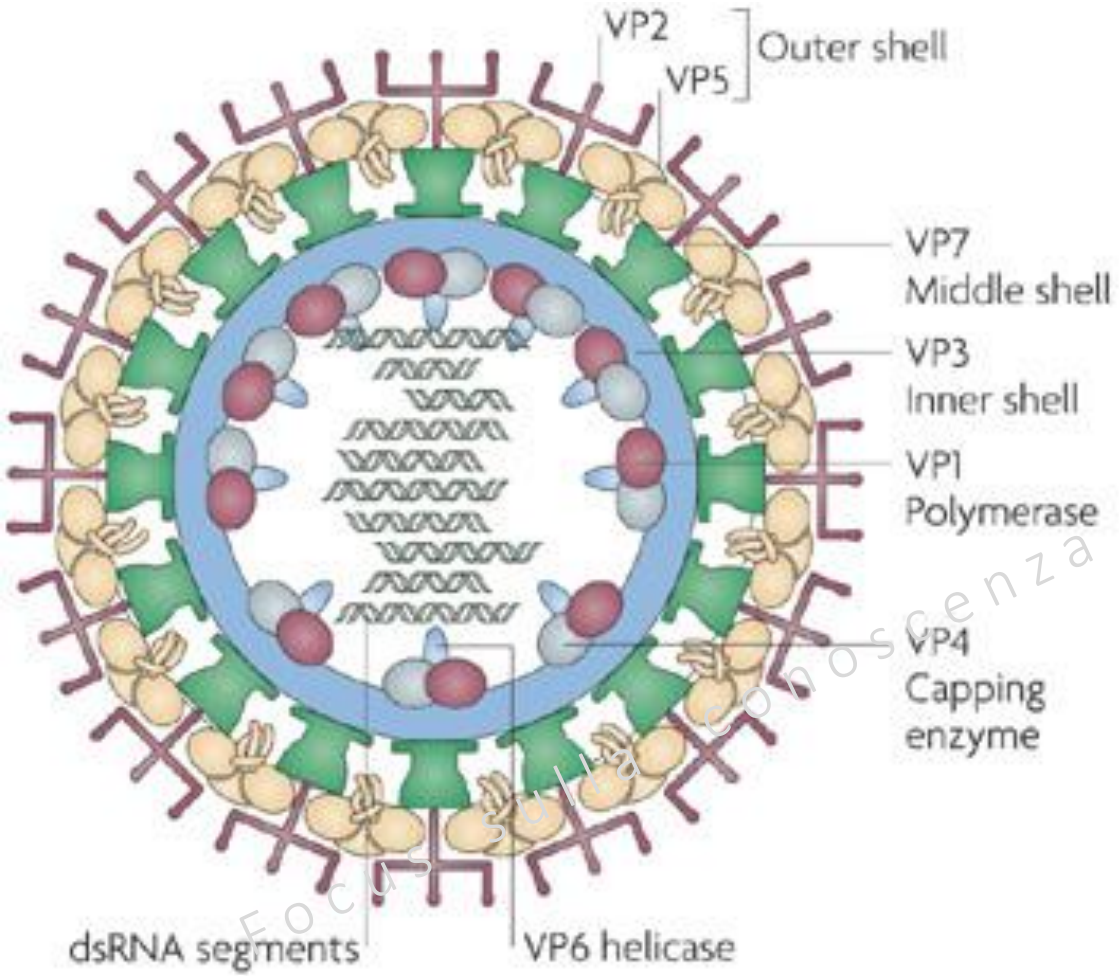
Eziologia



- VP7: Relevant to the identification of serogroup (BT virus)
- VP2: Relevant to the identification of the serotype of BTV

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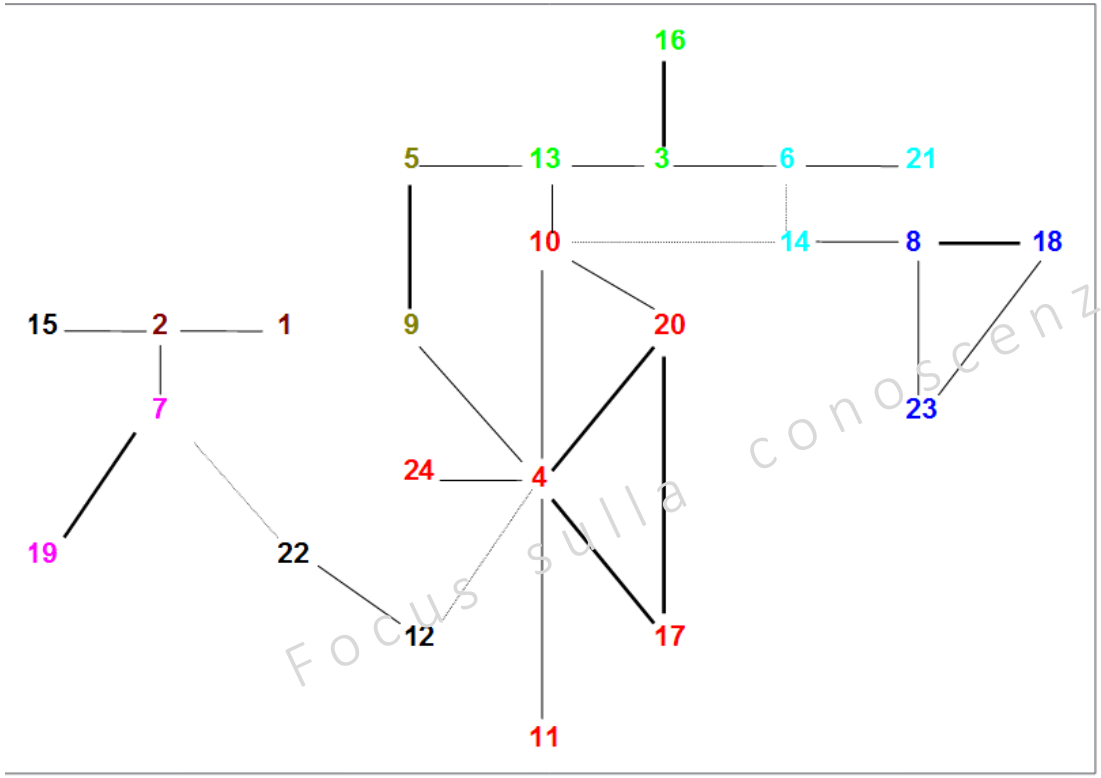
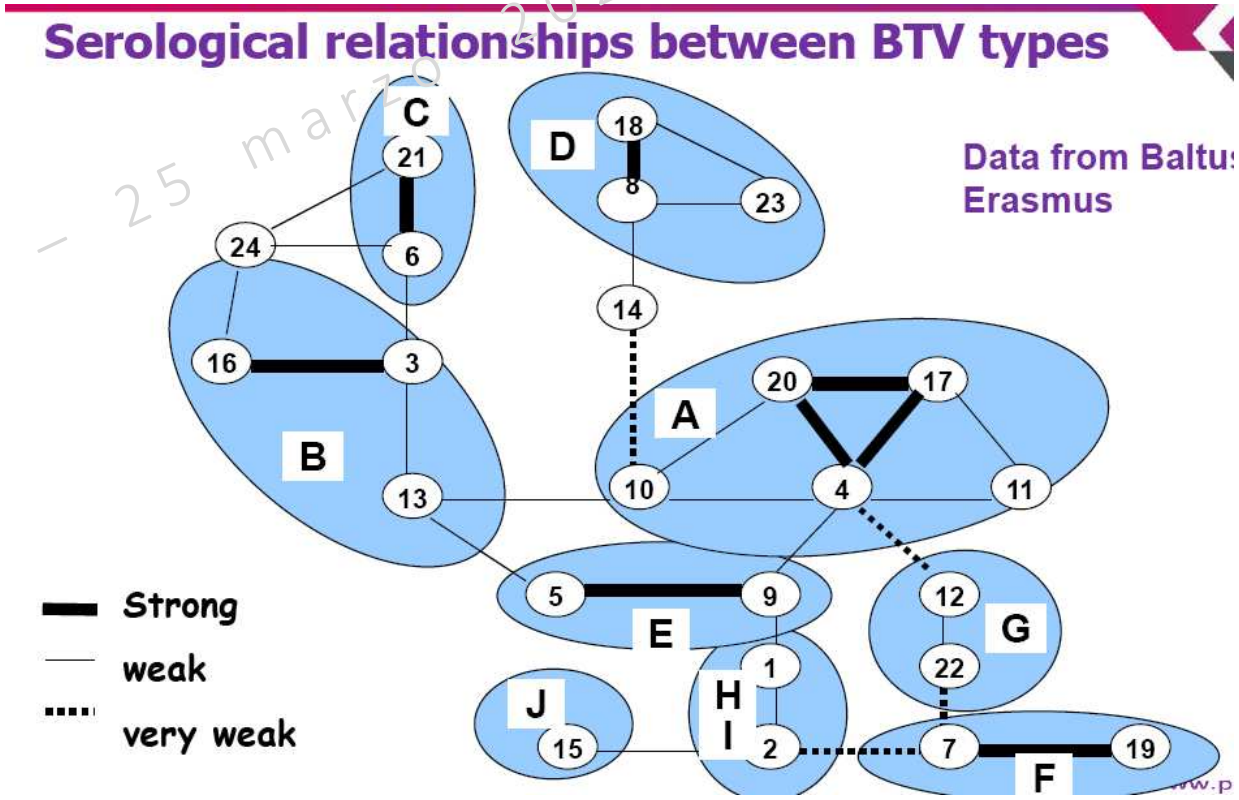
Source: Polly Roy et al. Nature Reviews Microbiology 7, 120-128 (2009)

Eziologia

- 24 traditional serotypes +7…
- partially correlated each other (in vitro cross-reactions and in vivo cross-protection)



Source: Erasmus, B.J., 1990, 'Bluetongue virus', in Z. Dinter & B. Morein (eds.), *Virus infections in ruminants*, pp. 227–237, Elsevier Science Publishers, Amsterdam



Source: Peter Mertens oral presentation

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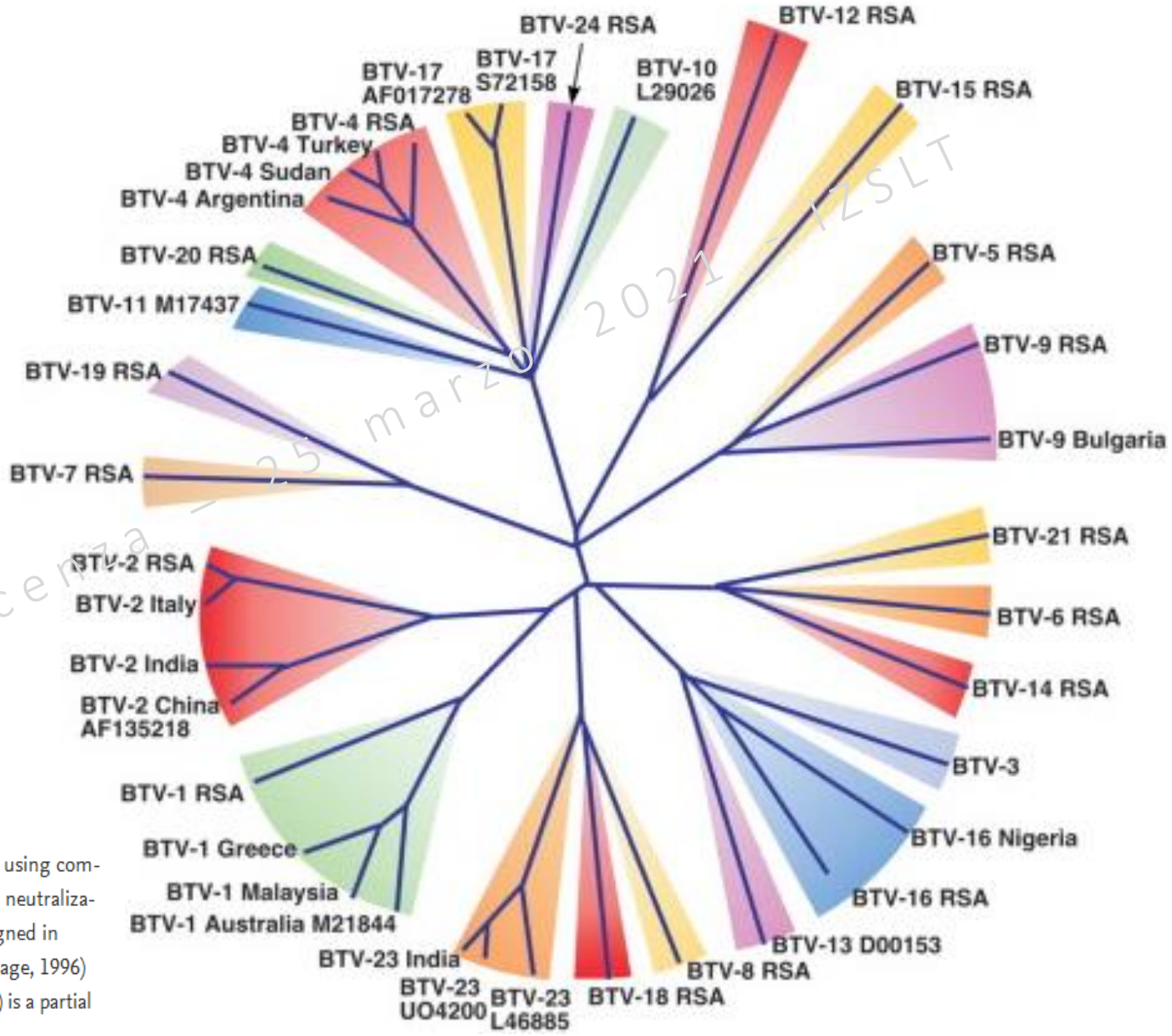
Eziologia



Source: Virus Taxonomy
2015, Pages 441, 443-569, 571-595, 597-605

shell). (Bottom) Phylogenetic tree for Bluetongue virus (Orbivirus type species) constructed using complete genome Seg2 sequences. Genome Seg2 codes for VP2, the larger outer CP and major neutralization (most variable) BTV antigen. Nucleotide sequences of genome Seg2 and Seg3 were aligned in Bioedit (Hall, 1999). The tree was prepared using Clustal X) and drawn with TreeView 1.5 (Page, 1996) (Courtesy of S. Maan, A. Samuel, N. Knowles and P.P.C. Mertens). The virus indicated by (p) is a partial sequence. Names corresponding to t h e abbreviations are from the "List of Species".

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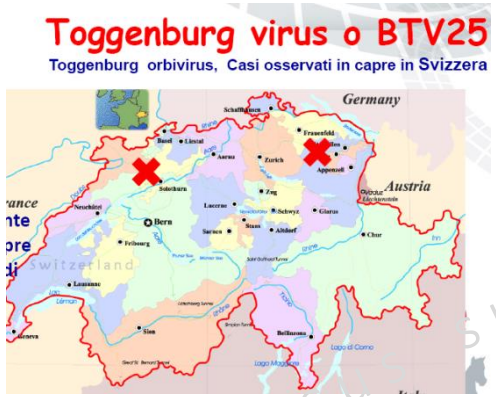
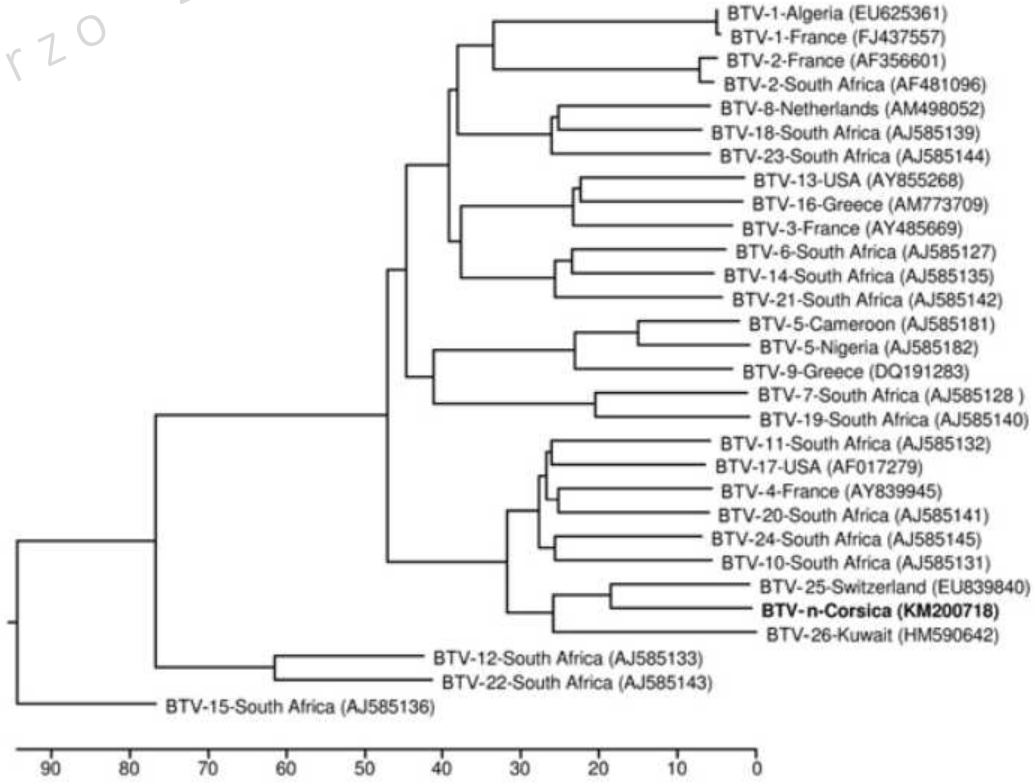
Nuovi sierotipi

- BTV 25 TOV goat clinically healthy
- BTV 26 Kuwait sheep&goat mild clinical signs
- BTV 27 Corsica goat clinically healthy





Phylogenetic tree of segment 2, (strains available in GenBank)
Source: Zientara, oral presentation

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Nuovi sierotipi

- not pathogen
- goat reservoir
- Not transmitted by Culicoides
- horizontal transmission between goats (direct contact)
- Long term persistence (in goats up to 19 months)

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Eziologia

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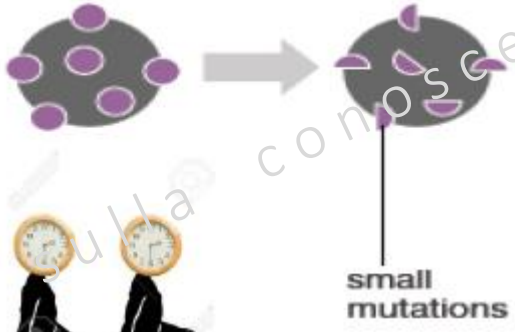
ISTITUTO G. CAPORALE TERAMO

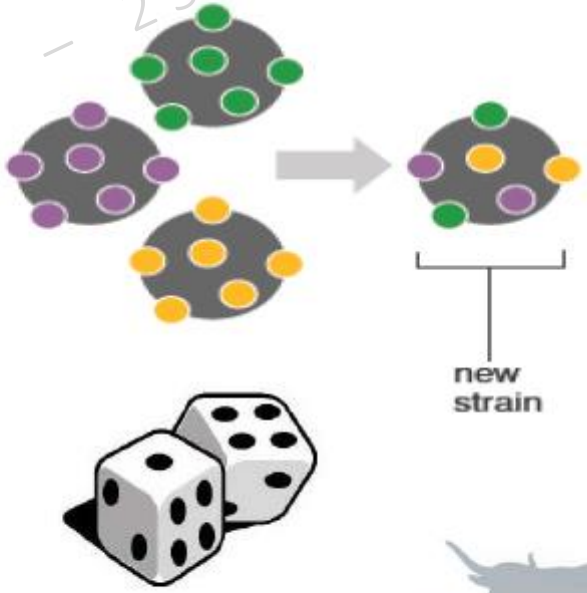
EVOLUTION

Mutation

Antigenic drift



Antigenic shift



Source: Lorusso, IZSAM

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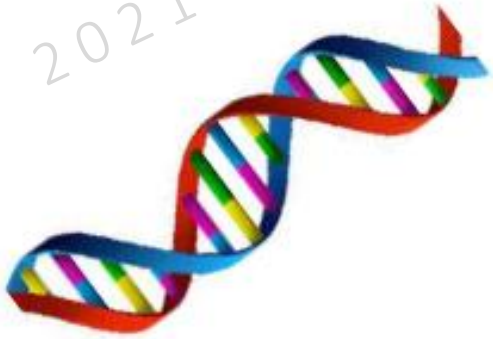
Eziologia

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



Source: Lorusso, IZSAM

DIFFERENT BTV-3!!!

BTV-3 TUN2016/Zarzis

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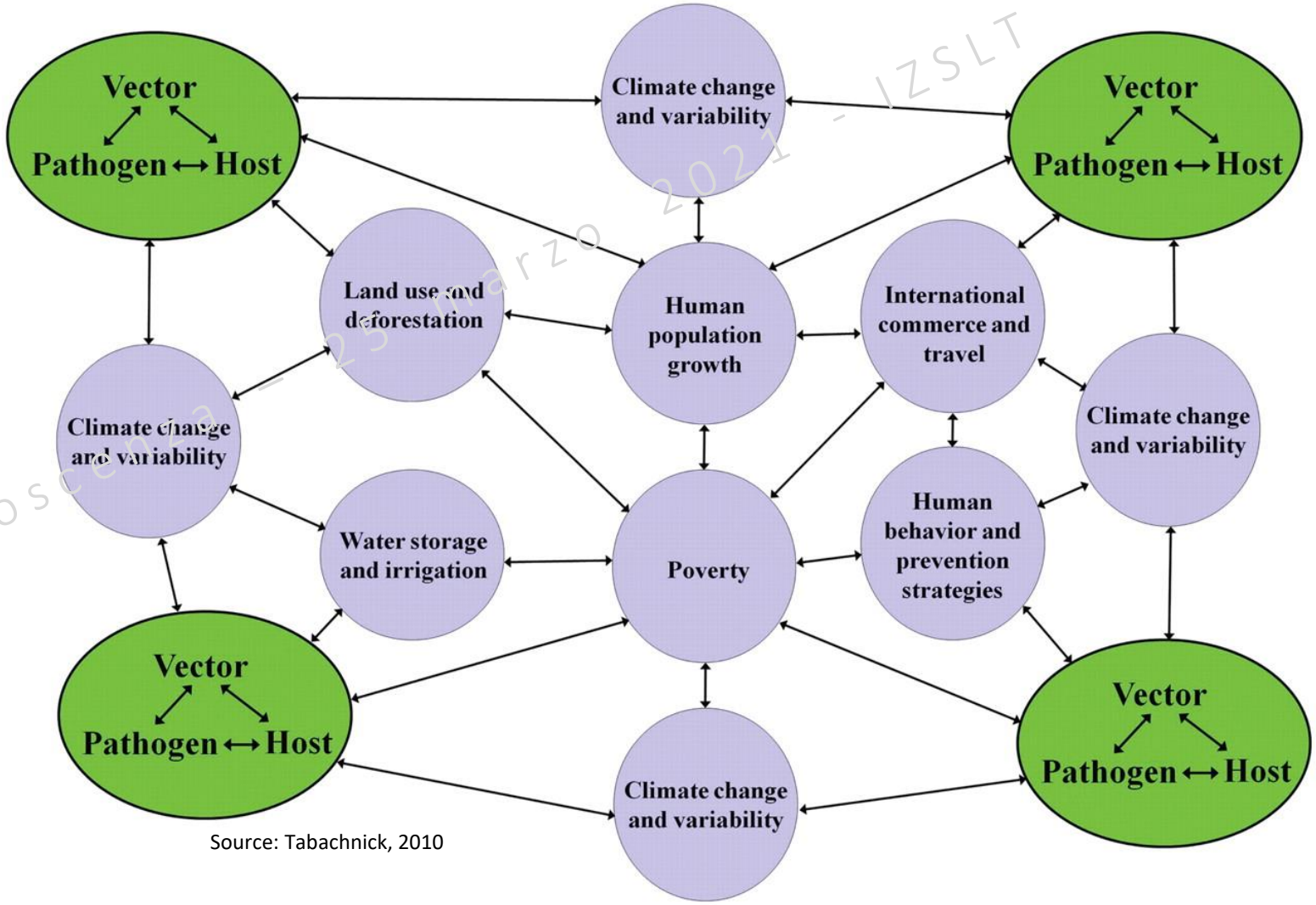
Natural Cycle

Interaction among

- **Vector**
- **Virus**
- **Ruminant host**

Environment

- Temperature
- Umidity
- Pedology soil type
- Predominant Winds



Source: Tabachnick, 2010

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EPISYSTEMS

- Distinct strains of BTV (virus topotypes) vectored by different species of *Culicoides* occur in specific regions of the world
- Topotypes of BTV and the vector species that occur within each episystem up to present times have been relatively stable, despite extensive and ongoing trade and movement of ruminants between individual episystems.

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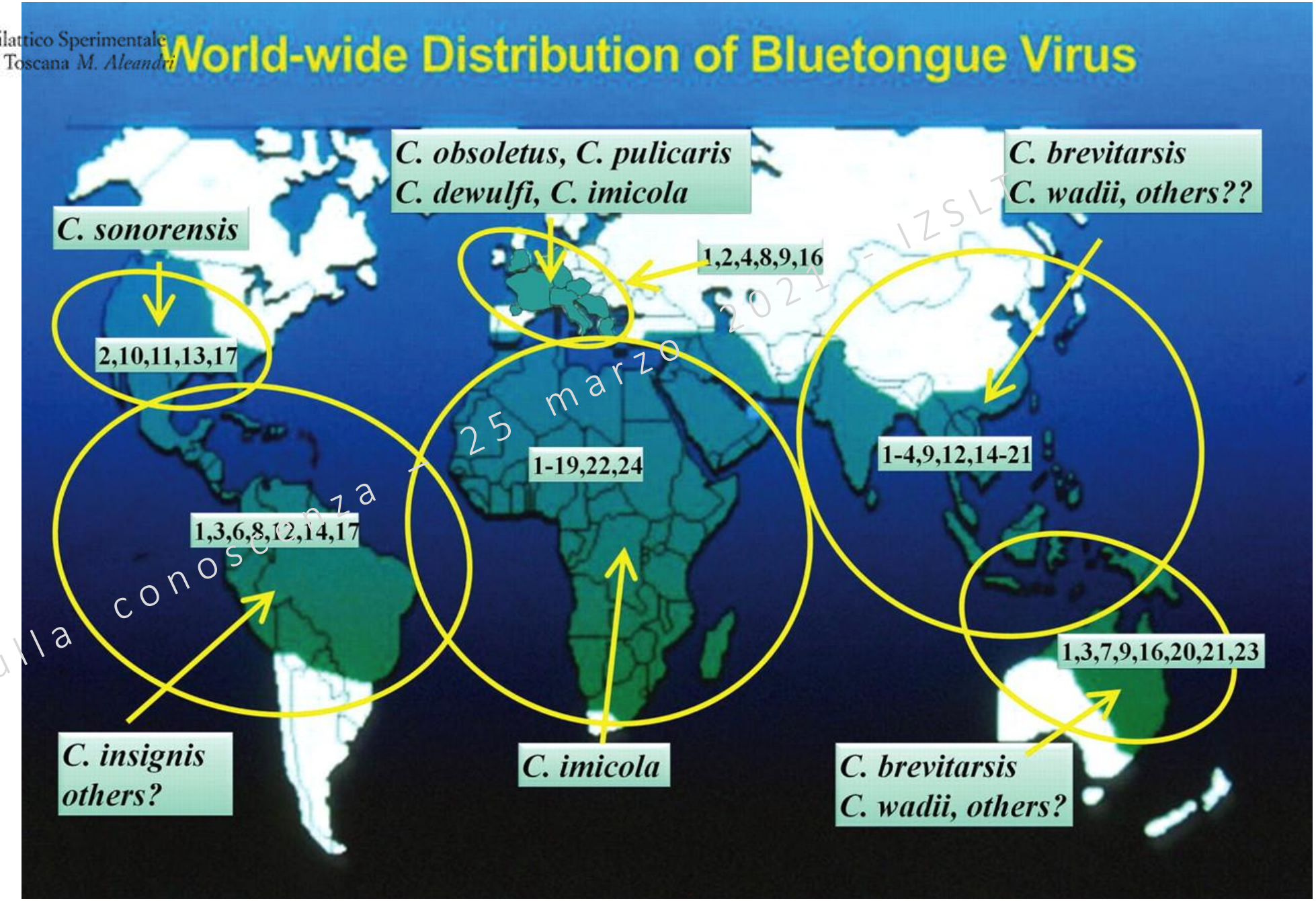

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BT Transmission

Photo Source: PLoS Biol. 2008 Aug; 6(8): e210.

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BT Transmission
Only culicoides ?

- Ticks, sheep keds (melophagus ovine)
- In utero
- Mechanical (surgical equipment and needles)
- Horizontal (contact)
  - demonstrated in BTV 2, 8, 26
  - hypotized in BTV25
- Venereal?
- ingestion of infected material (carnivores or calves)

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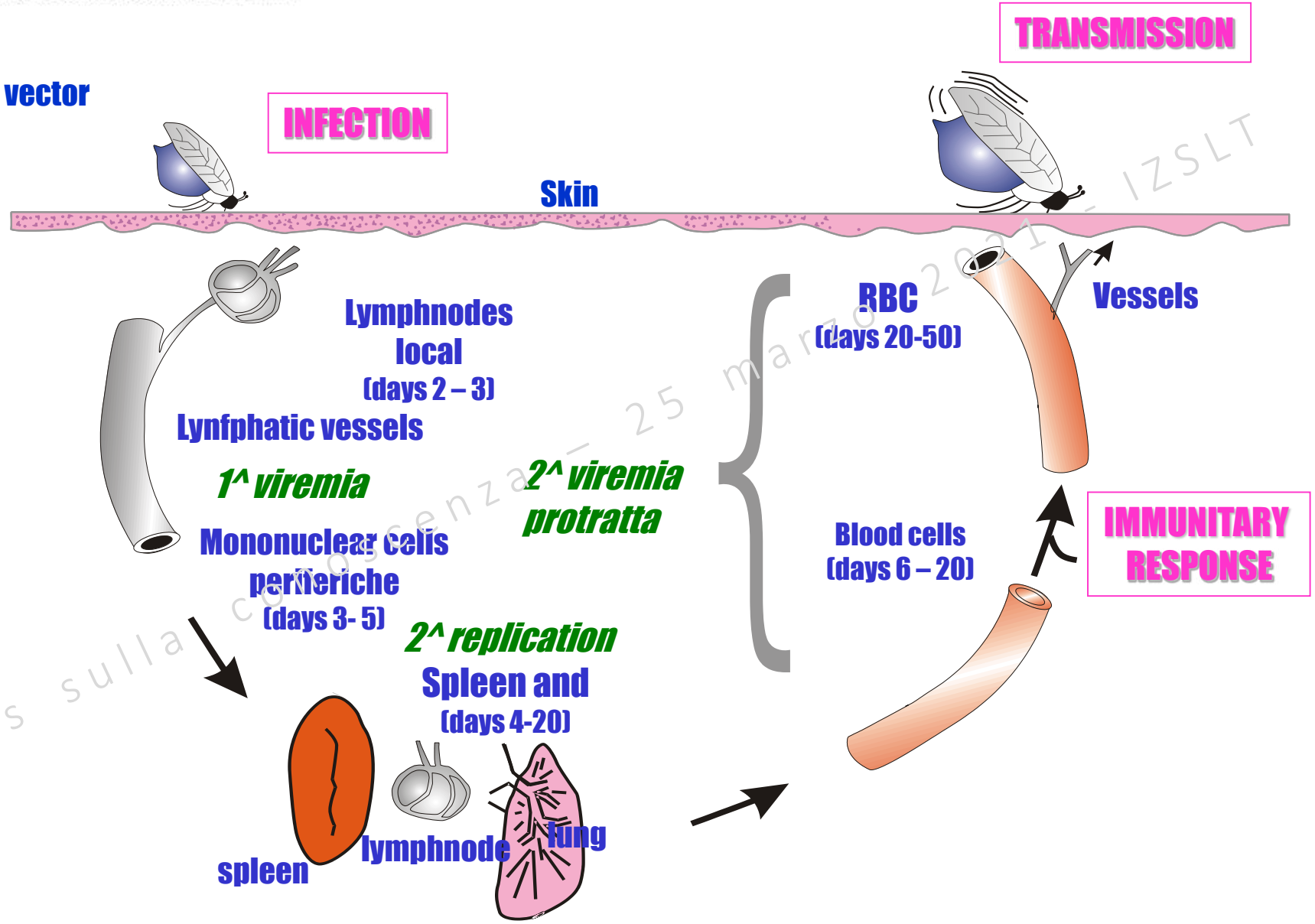
PATHOGENESIS IN BOVINE



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Source:
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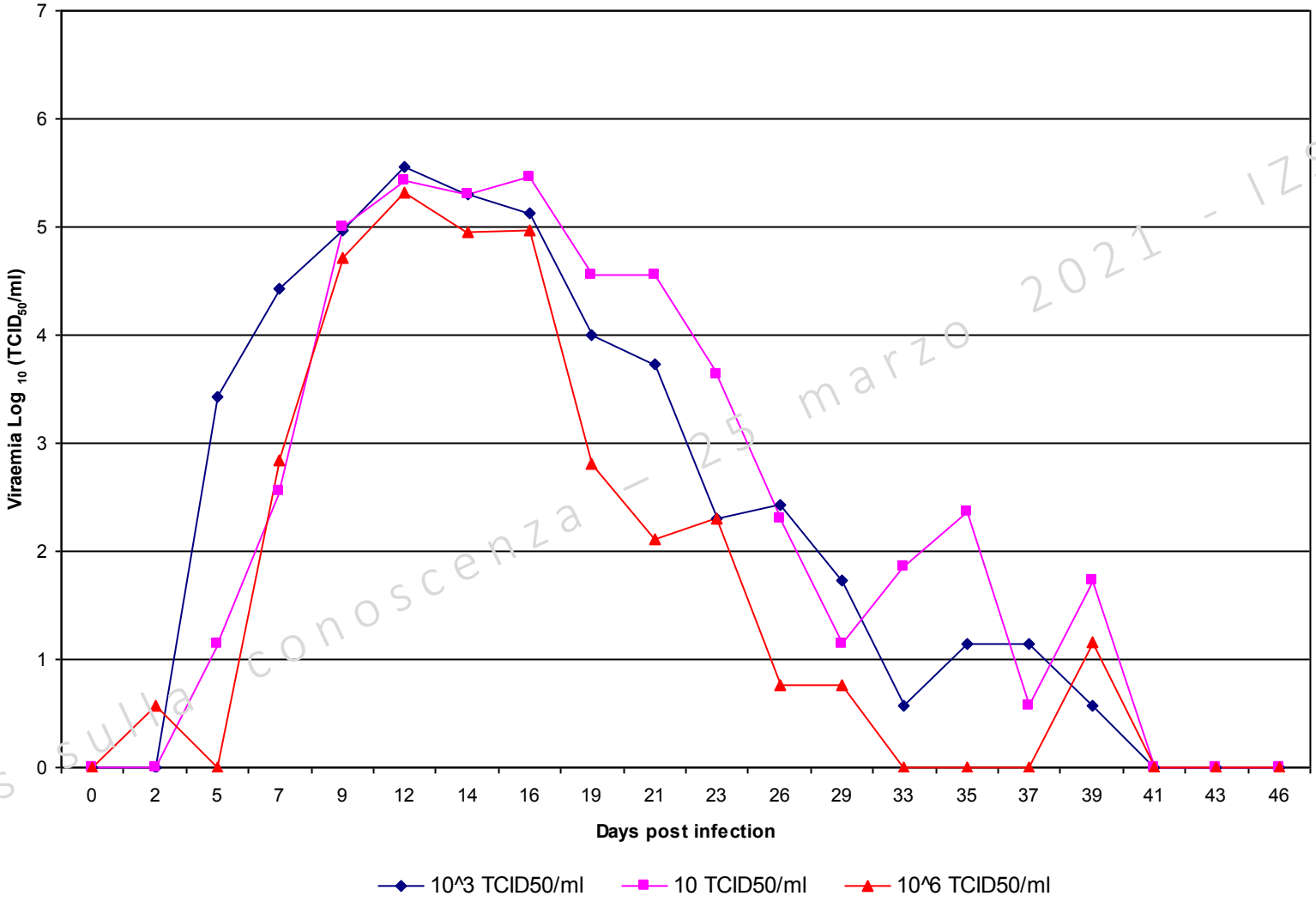
Viraemia in bovine



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Source: IZSAM

Mean Viraemia titers in bovine challenged with different dose of BTV-8

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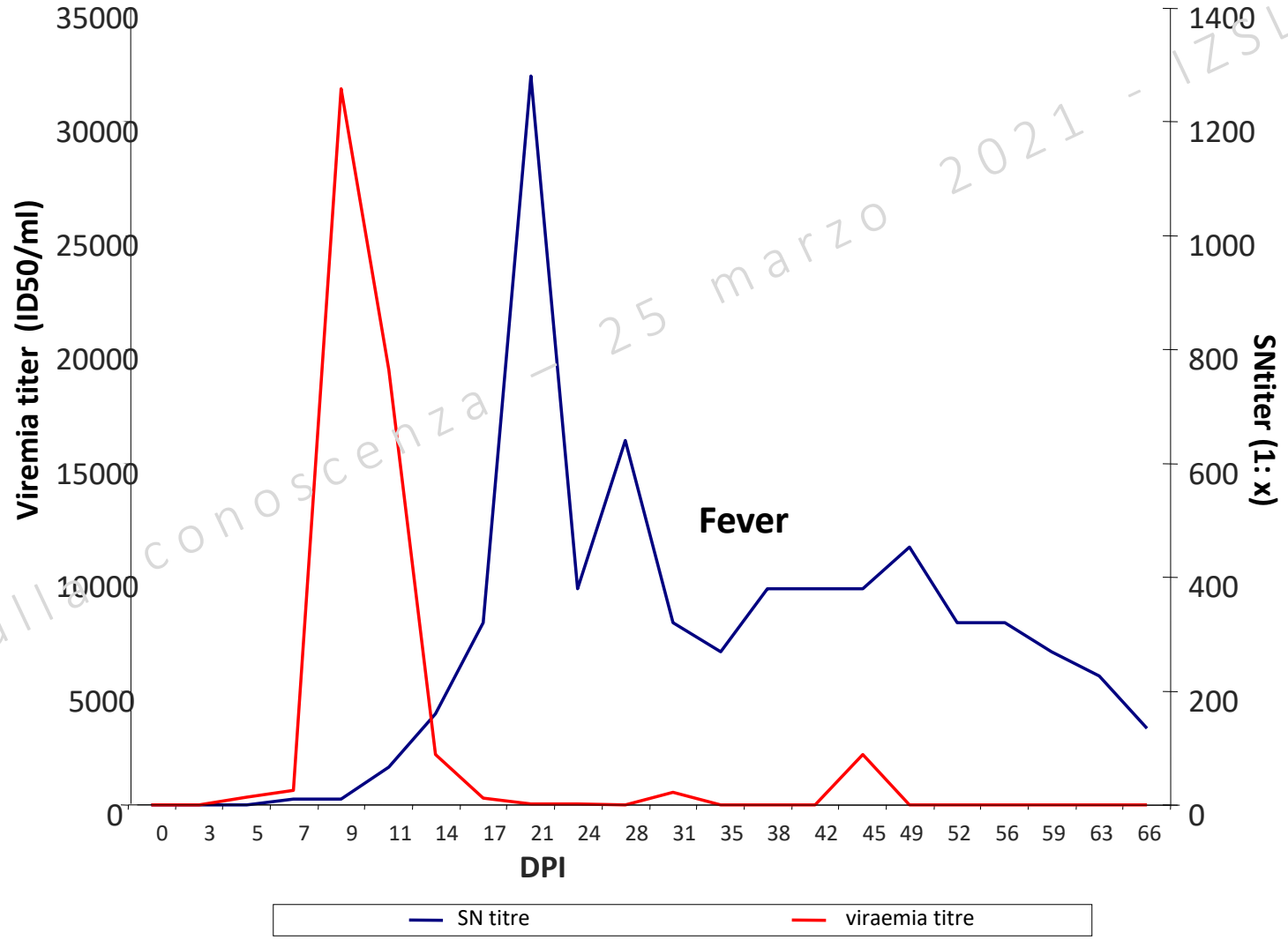
PATHOGENESIS IN SHEEP



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Source: IZSAM

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BT Pathogenesis
Interaction virus-host

Strictly associated to cells

Protected from N Ab

Virus in RBC fold of membrane and platelet

It provokes the coagulation process -> damage endotelium

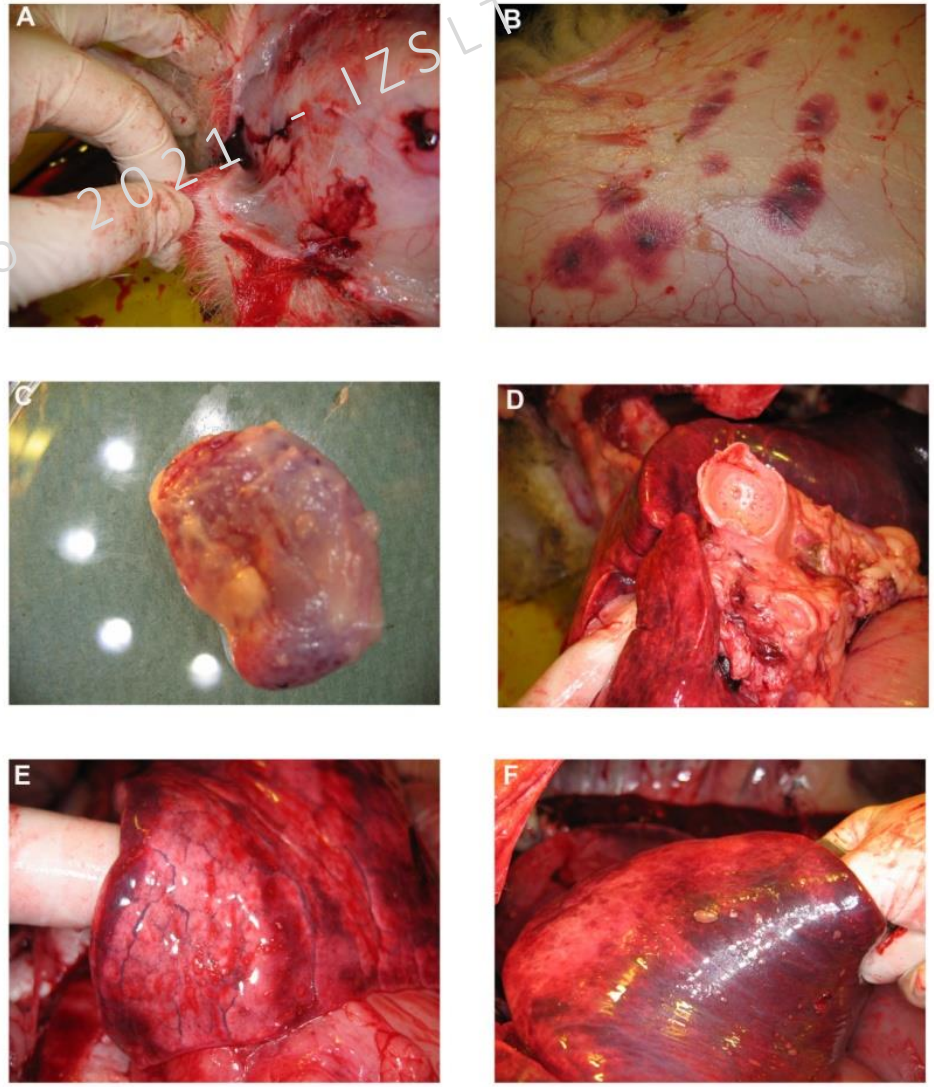
severe oedema in the subcutaneous layer of the facial skin **(A)**

severe haemorrhages on the subcutaneous aspect of the skin **(B)**

Both prescapular lymph nodes were markedly enlarged and haemorrhagic **(C)**

Trachea and bronchus)contained serous fluid or froth **(D)**

interstitial oedema was observed in the lung tissue **(E-F).**



2021 - IZSLT
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Source: Involvement of the skin during bluetongue virus infection and replication in the ruminant host
Darpel et al, Veterinary Research 2012 **43**:40

Morbidity/Mortality: Sheep

- Sheep
  - Severity of disease varies
    - Breed
    - Strain of virus
    - Environmental stress
  - Morbidity
    - As high as 100%
  - Mortality
    - Usually 0 to 30%

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Photo: A flock of sheep. USDA ARS

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Morbidity/Mortality: Other Species

- Cattle, goats
  - Morbidity: up to 5%
  - Death is rare
- Deer, antelope
  - Severe infection
  - Morbidity
    - Up to 100%
  - Mortality
    - 80 to 90%

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Photo: (Top) Alice Welch/USDA; (Middle): www.wikimedia-commons.org ; (Bottom): Bob Nichols/USDA

Clinical outbreaks – data 2000-2002

REGION	Outbreaks	No. of sheep in diseased flocks	No. of diseased animals	No. of dead animals	No. of killed animals
2000-2001					
CALABRIA	589	89,166	15,676	1,011	16,485
SARDINIA	6,264	1,360,614	246,908	46,993	213,863
SICILY	16	1,760	175	106	94
TOTAL (2000-2001)	6,869	1,451,540	262,759	48,110	230,442
2001-2002					
BASILICATA	63	4,766	152	16	-
CALABRIA	427	52,722	10,176	236	9,877
CAMPANIA	1	64	3	2	-
LATIUM	62	23,040	431	69	356
SARDINIA	6,090	1,294,365	239,178	72,502	159,636
SICILY	6	853	29	29	-
TUSCANY	158	33,988	693	119	560
TOTAL (2001-2002)	6,807	1,409,798	250,662	72,973	170,429

Source: IZSAM

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Numbers 2015

Species	Susceptible	Cases	Deaths	Morbidity	Mortality	Fatality
BTV1	**204125**	**12168**	**3070**			
Sheep	180911	10957	3062	6.1%	1.7%	27.9%
Cattle	20725	1127	4	5.4%	0.0%	0.4%
Goats	2489	84	4	3.4%	0.2%	4.8%
BTV4	**99348**	**6927**	**1217**			
Sheep	45748	3733	470	8.2%	1.0%	12.6%
Sheep / goats	21921	980	735	4.5%	3.4%	75.0%
Cattle	31058	2207	12	7.1%	0.0%	0.5%
Goats	621	7	0	1.1%	0.0%	0.0%
BTV8	**262992**	**3603**	**484**			
Cattle	218451	2041	4	0.9%	0.0%	0.2%
Sheep	17952	745	214	4.1%	1.2%	28.7%
Sheep / goats	26540	812	265	3.1%	1.0%	32.6%
Goats	49	5	1	10.2%	2.0%	20.0%

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Source: OIE Wahis

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IMPACT OF BT

Depends on

- Condition for virus persistence
  - Wheather
  - Density susceptible animal
- Importance of trade
  - Mainly sending
  - Or receiving animals
- Virus serotype
- Susceptible population
  - Mainly ovine
  - Mainly bovine

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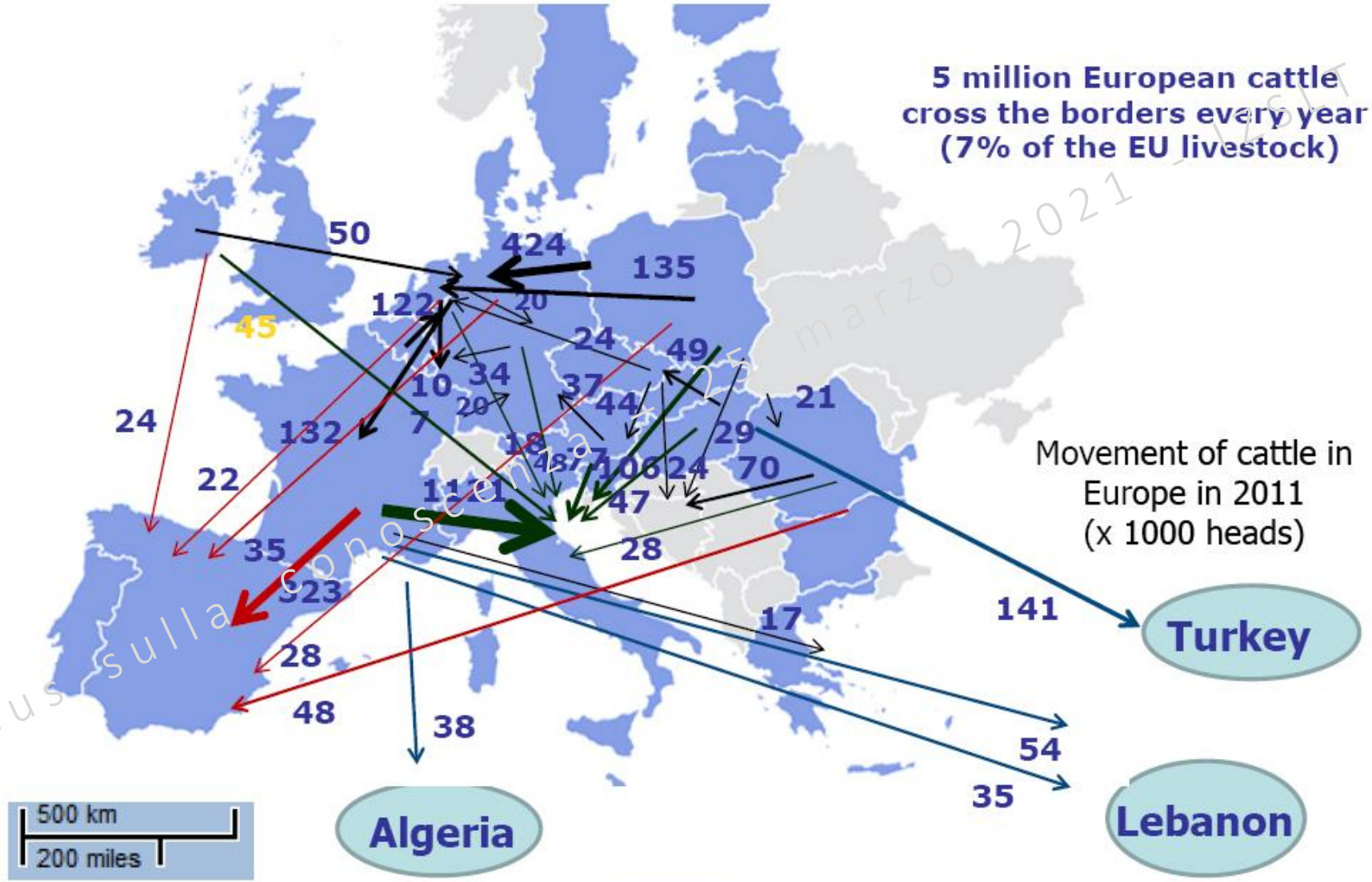
Impact: Trade movements within the EU



Source: GEB-Animal Breeding Institute as per Eurostat

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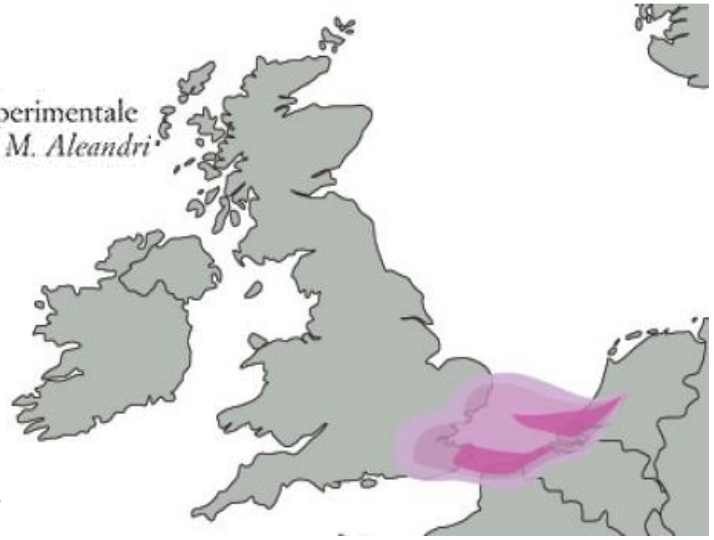

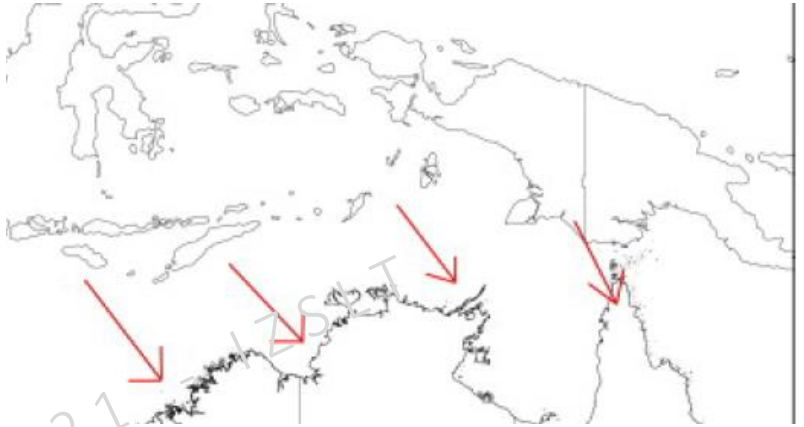

Where from:

- **Animal movements**
- Transportation of animal products ?
- Inanimate (aeroplanes? ships?)
- **Passive windborne transport of infected vectors**
- **Anthropogenic drivers ?**

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Source: www.earthobservatory.nasa.gov/NaturalHazards

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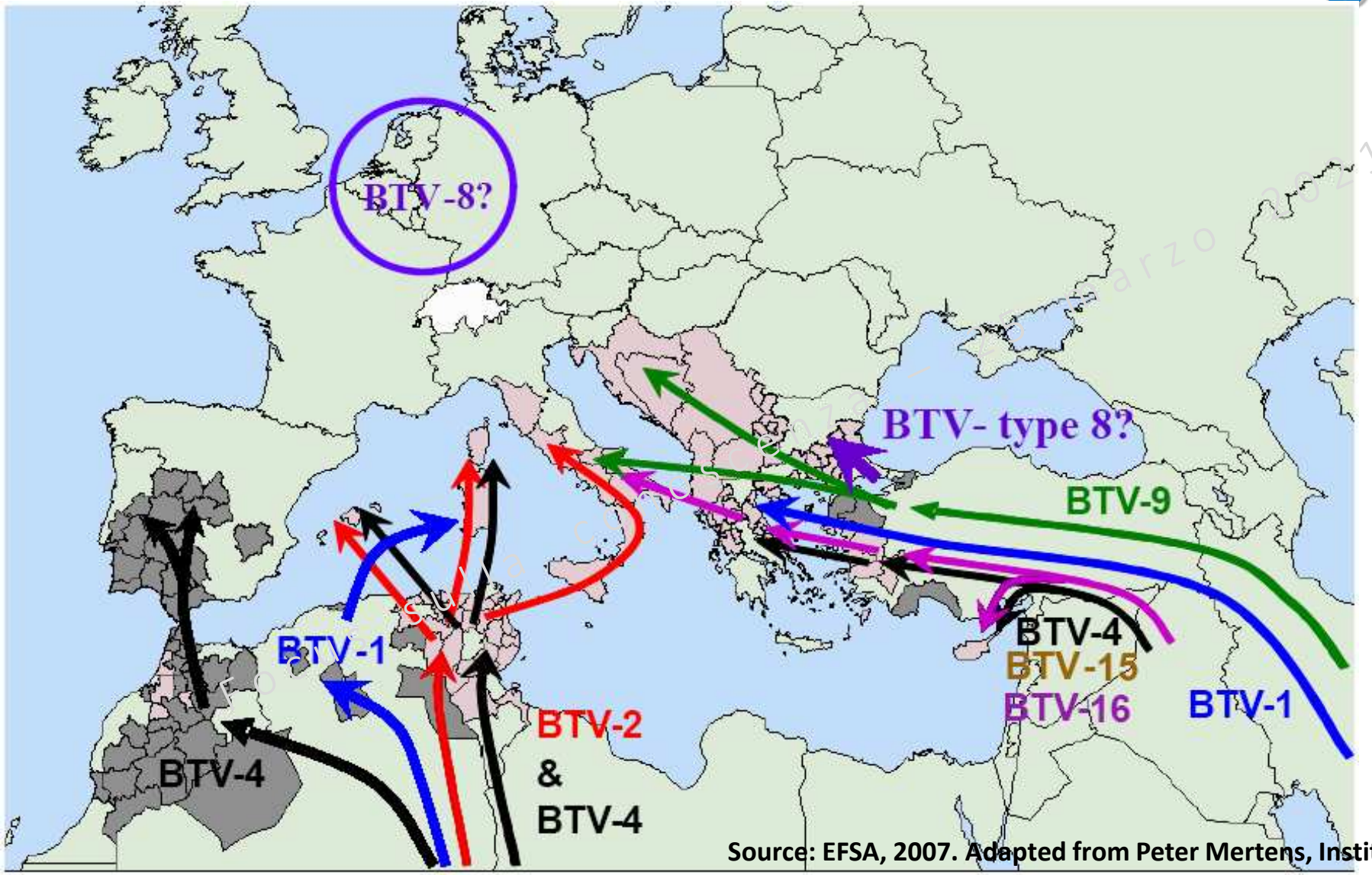
POSSIBLE ROUTES OF INTRODUCTION OF BTV IN EUROPE SINCE 1998



Source: EFSA, 2007. Adapted from Peter Mertens, Institute for Animal Health

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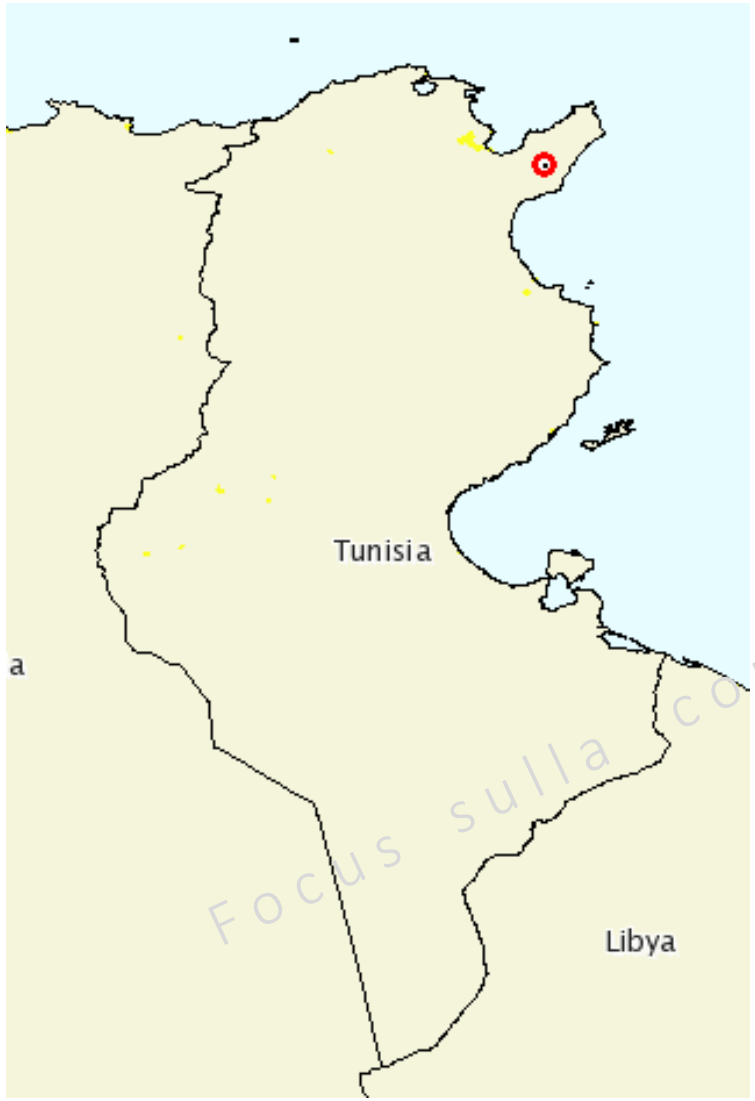
BTV3…



Outbreak 1	**Beni khalled, Hannous, Beni khalled, NABEUL**
Date of start of the outbreak	01/11/2016
Outbreak status	Resolved (31/01/2017)
Epidemiological unit	Farm

Affected animals

Species	Susceptible	Cases	Deaths	Destroyed	Slaughtered
Cattle	8	0	0	0	0
Goats	7	0	0	0	0
Sheep	46	1	0	0	0

Affected population	This is a Barbarine ewe over three years old. It showed dejection, fever, loss of weight, salivation, facial and submandibular oedema, nasal discharge, tachycardia and dyspnoea.
Summary of outbreaks	**Total outbreaks: 1**

Total animals affected

Species	Susceptible	Cases	Deaths	Destroyed	Slaughtered
Cattle	8	0	0	0	0
Goats	7	0	0	0	0
Sheep	46	1	0	0	0

Outbreak statistics

Species	Apparent morbidity rate	Apparent mortality rate	Apparent case fatality rate	Proportion susceptible animals lost*
Cattle	0.00%	0.00%	-	0.00%
Goats	0.00%	0.00%	-	0.00%
Sheep	2.17%	0.00%	0.00%	0.00%

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Arthropod vector

Class: Insecta

Order: Diptera

Suborder : Nematocera

Family: Ceratopogonidae

Genus: *Culicoides*

AMONG 1400 SPECIES EXISTING

< 3%

ARE CERTAIN OR SUSPECTED VECTORS OF BT

Source: IZSAM

Focus sulla conoscenza – 25 marzo 2021 – IZSLT

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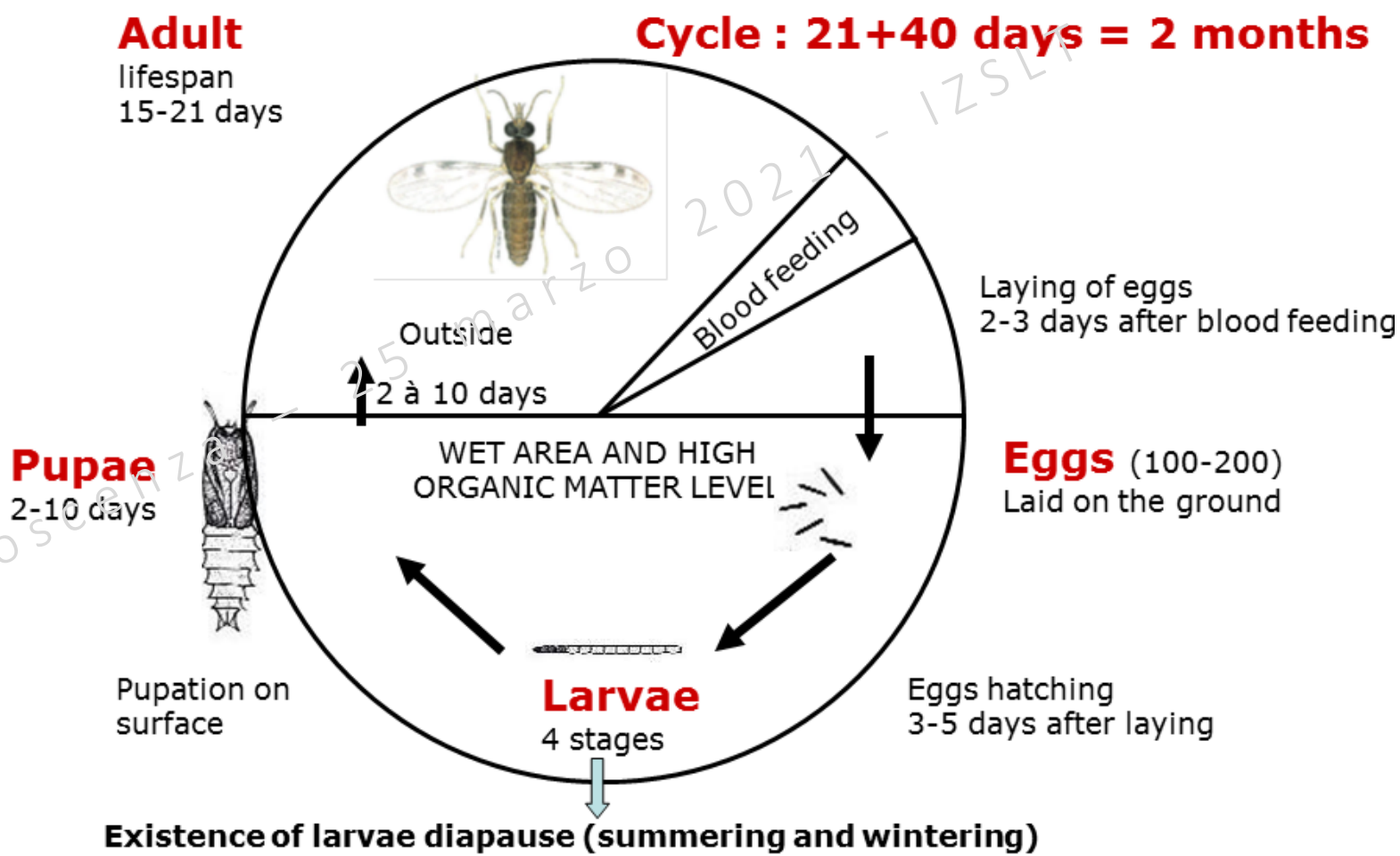
Life cycle of *Culicoides imicola*



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Source: //www.aboutbluetongue.com/Pages/InsectVector.aspx

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Vector movement

Adults *Culicoides* :

- actively (some hundreds meters)
- passively through winds:
  - Surface winds-> 10-50 Km
  - Altitude winds-> hundreds Km

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If virus reaches salivar glands

- % infection in midges depends on viremia
  - *C. imicola*: 24% con BTV6; 30% con BTV3
- Midges are infected all life long (3-6 weeks)
- Every midge could infect an animal every 3 days
- No transovarial nor transtadial transmission

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CULICOIDES

Main vectors:

- *C. imicola (Africa, Mediterranean basin)*
- *C. obsoletus (Cyprus, 1977; Bulgaria, 1999; Italy, 2002; Northern Europe, 2006)*
- *C. pulicaris (Italy, 2002)*
- *C. dewulfi (Northern Europe, 2006)*
- *C. brevitarsis, C. fulvus, C. oxystoma, C. peregrinus (Australia)*
- *C. variipennis, C. insignis (North America)*

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LARVAL HABITAT

Midges have different reproduction habitats according to their species:

- *C. imicola* needs terreno umido (fango) esposto al sole (argilla)
- *C. obsoletus* breeds in shadowy areas (woods)
- *C. chiopterus dewulfi* breeds in feces (bovine or horses)

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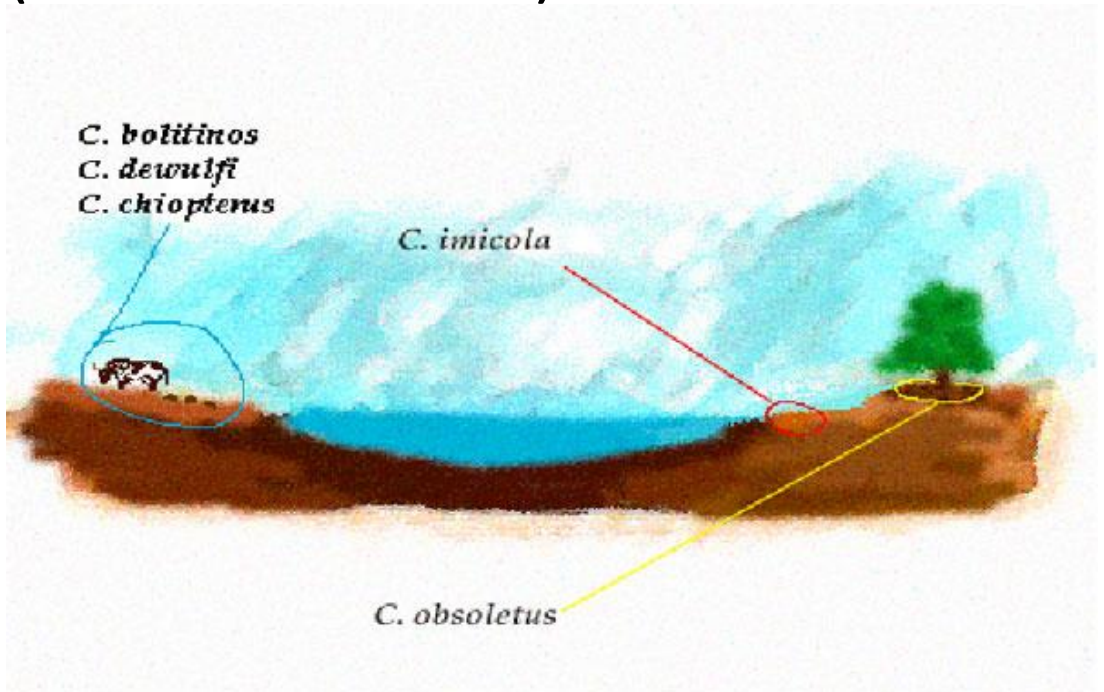

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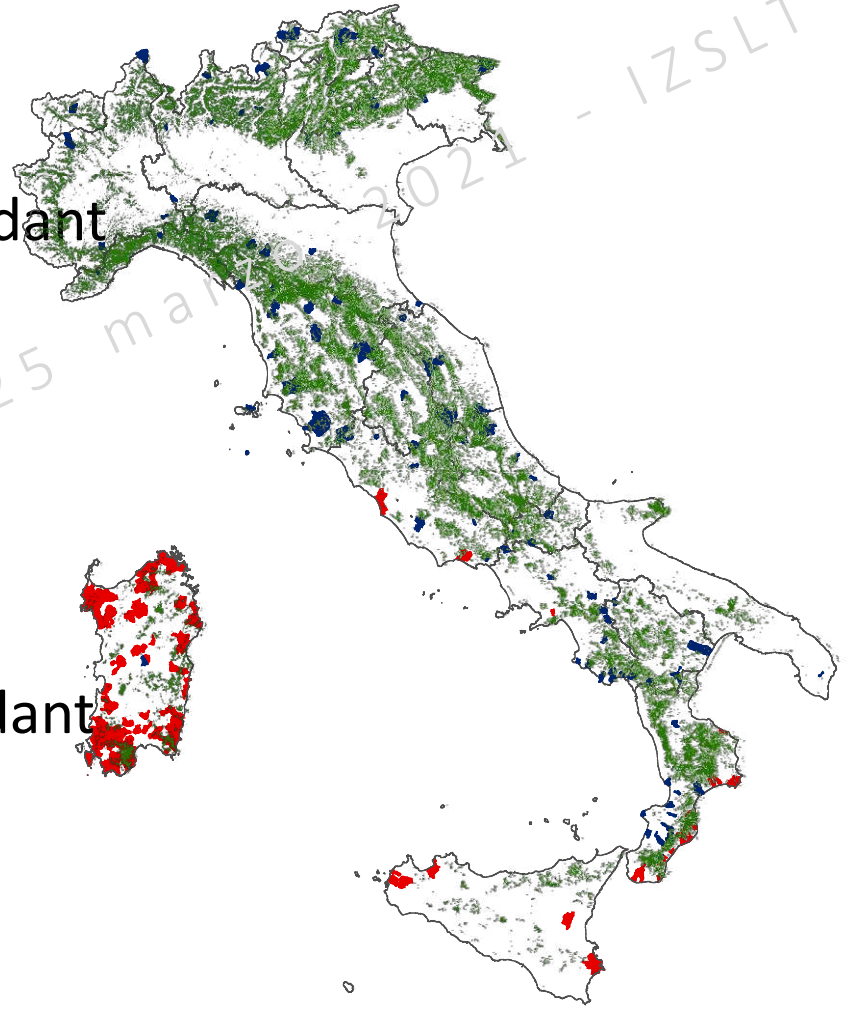
Obsoletus Complex vs C. imicola forest coverage

- *C. imicola*
  100 catches more abundant
- Obsoletus Complex
  100 catches more abundant



Source:
IZSAM

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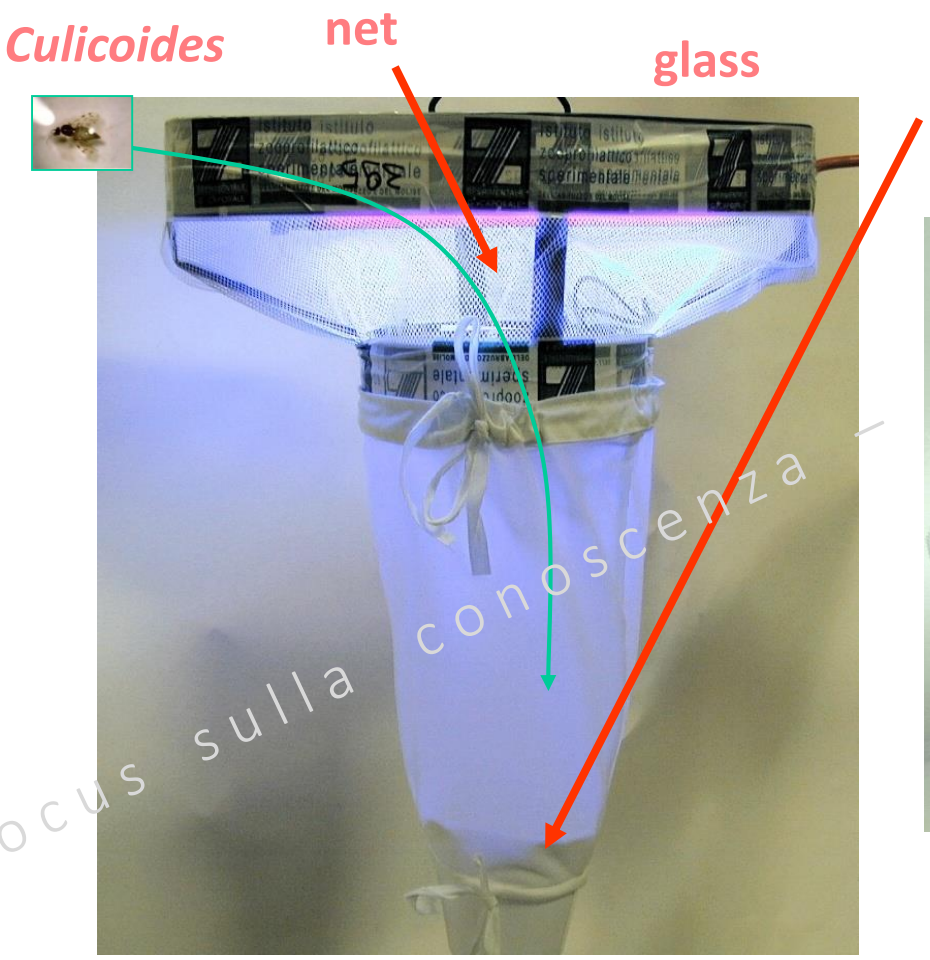
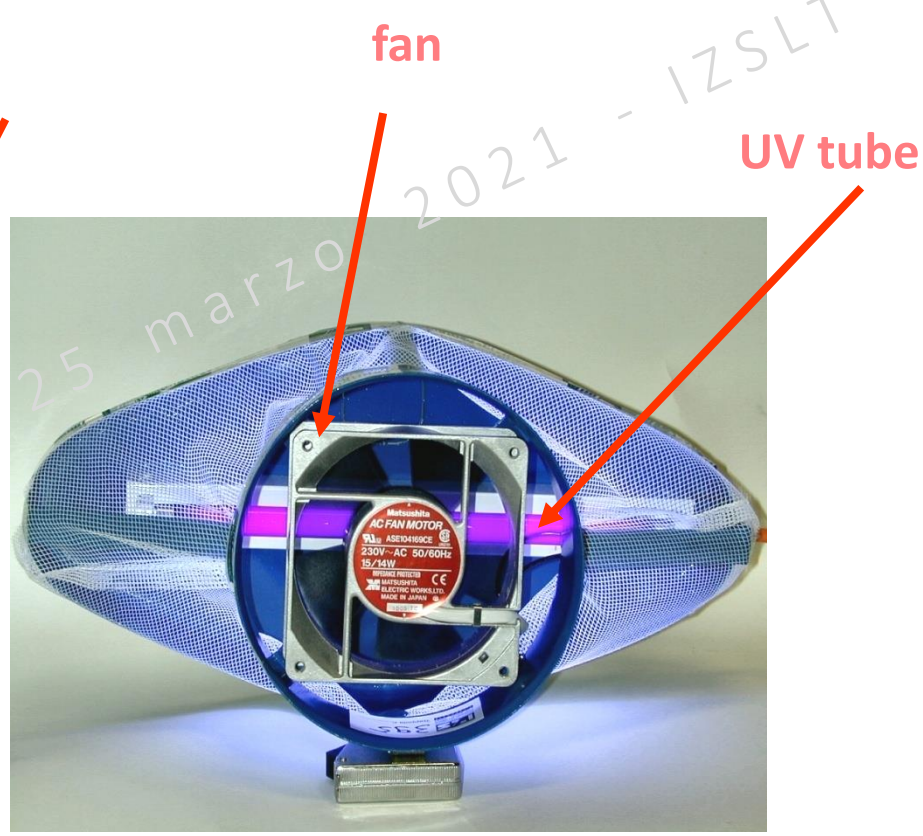
BLACK LIGHT TRAP





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Source:
IZSAM

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Where to put the trap ?

Source: IZSAM

Sardegna 2000: 1st outbreak BT (20,000 Imicola 1 night). Trap needs to stay close to the restaurant…

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Where to put the trap ?

Puglia. Few Imicola/night

Source:
IZSAM

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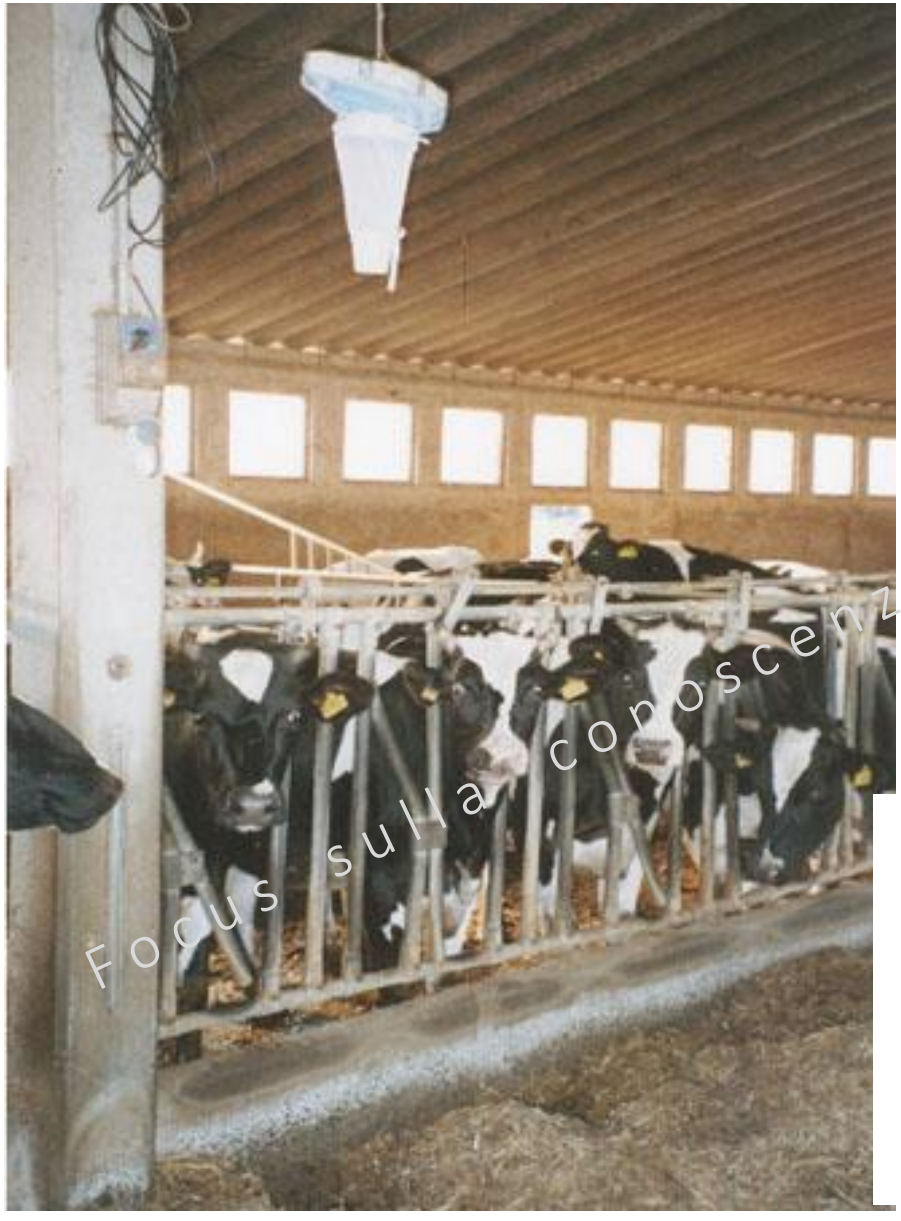
Obsoletus complex



- Captured inside cattleshed
- Can live more than 3 months
- Resistant to low temperature
- Active also during daylight

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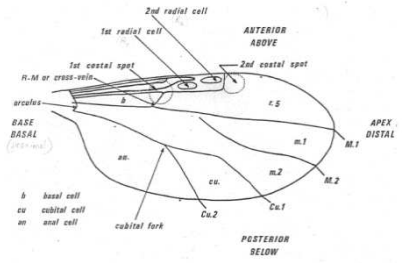
› Med Vet Entomol. 2018 Mar;32(1):70-77. doi: 10.1111/mve.12260. Epub 2017 Aug 22.

Indoor and outdoor winter activity of Culicoides biting midges, vectors of bluetongue virus, in Italy

A Magliano [1], P Scaramozzino [1], S Ravagnan [2], F Montarsi [2], G DA Rold [2], G Cincinelli [3], A Moni [4], P Silvestri [5], A Carvelli [1], C DE Liberato [1]

Catch analysis: morfological exam

midges → *CULICOIDES* →



- *C. imicola*
- others

- *Obsoletus Complex*
  - *PCR*
  - *C. obsoletus s.s.*
  - *C. scoticus*
  - *C. montanus*
- *Pulicaris Complex*
- *C. dewulfi*
- *C. chiopterus*

Source: IZSAM

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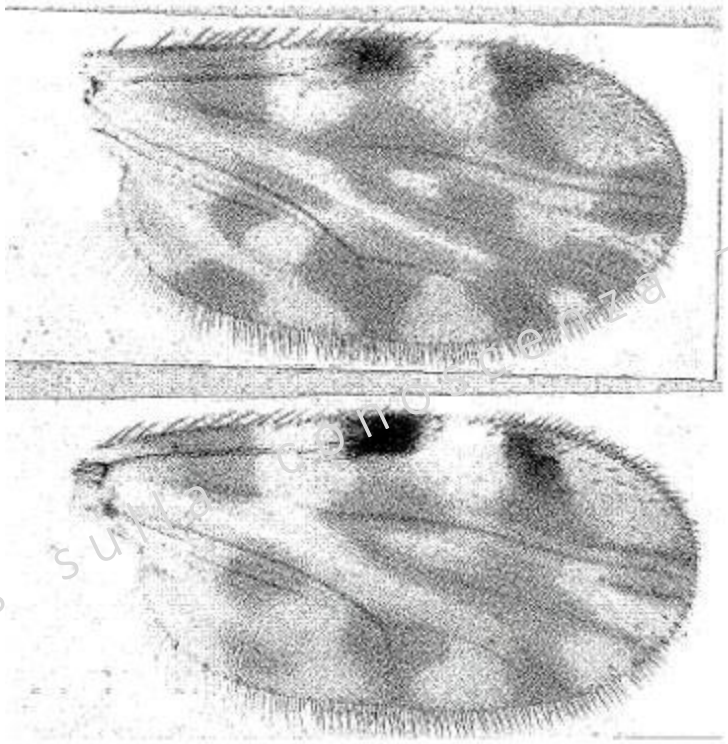
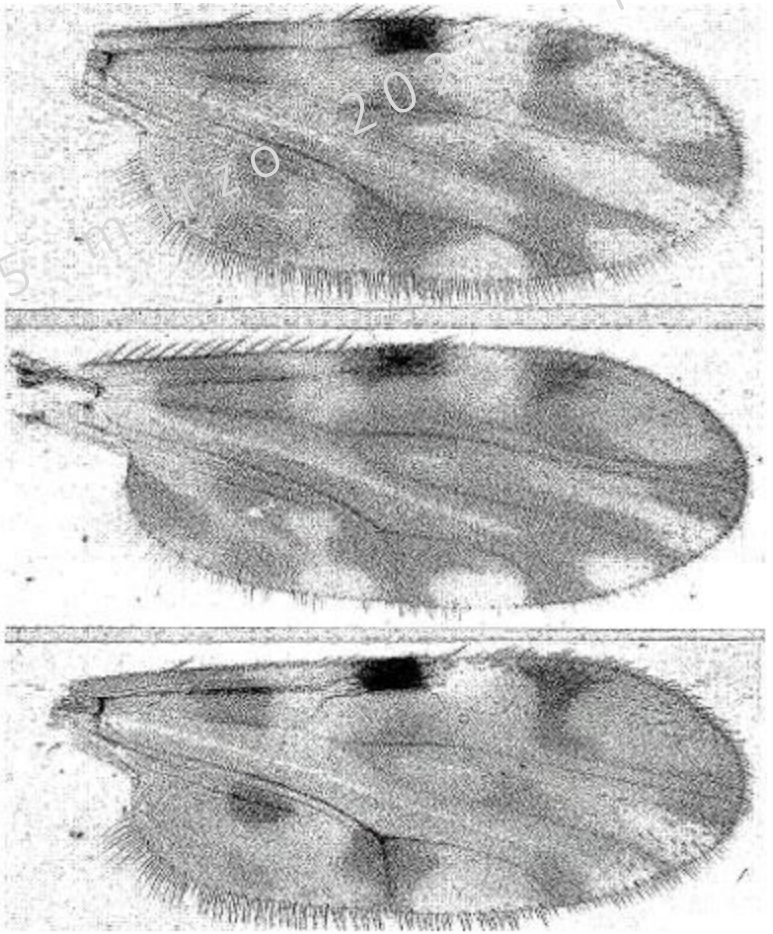
How identify Culicoides? (wings of 5 different spp)





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And in winter ?

Focus sulla conoscenza
– 25 marzo 2021 - IZSLT

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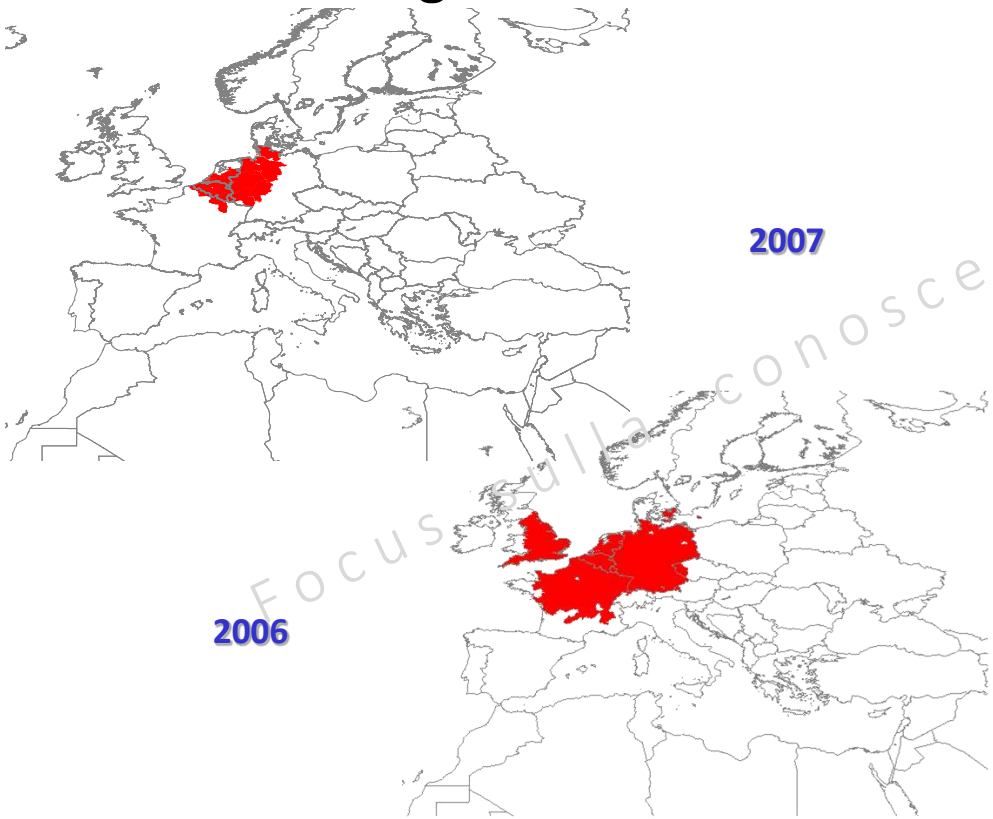
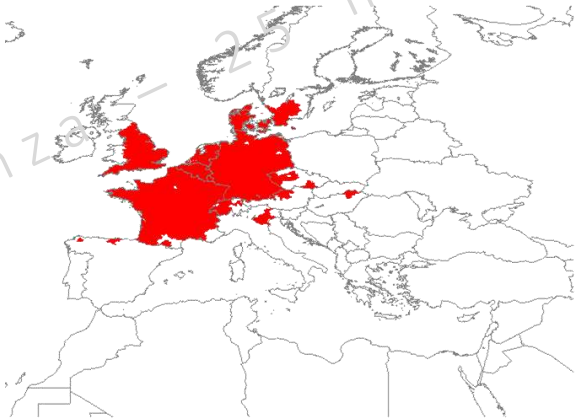
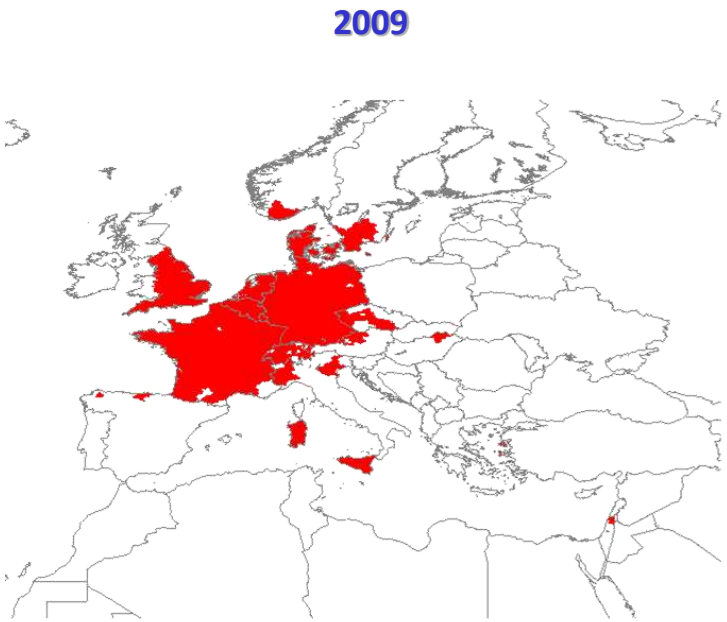
BTV 8 OCCURRENCE IN EUROPE FROM 2006 TO 2009

- In August 2006, a new BTV serotype (BTV-8) was detected in the Netherlands, France, Belgium and Germany
- The origin and mean of introduction are still unknown







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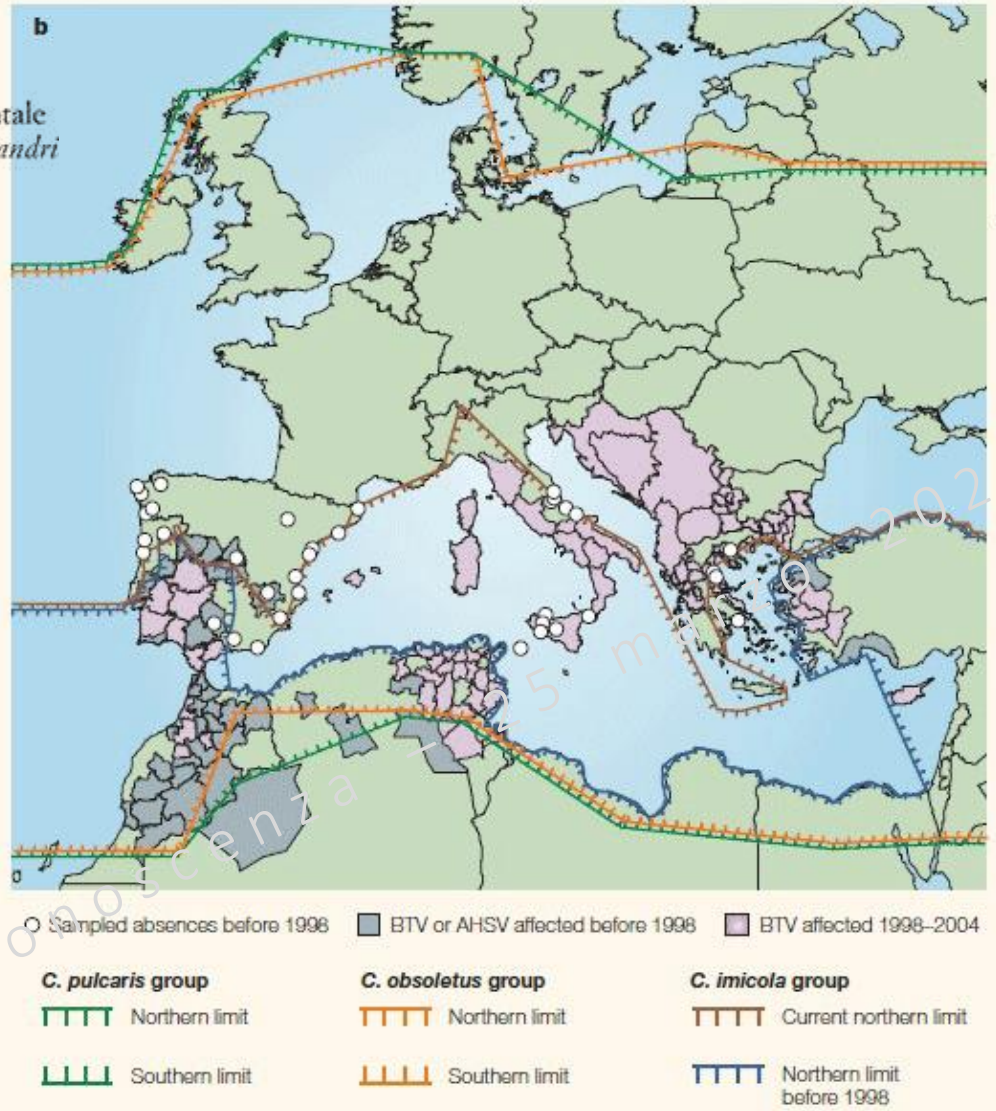

Figure 1 | **Distribution of BTV and *Culicoides* vectors. a** | Map showing the countries where *Culicoides imicola* — the major Old Word bluetongue virus (BTV) vector — has been recorded (indicated in dark green). The current distribution limits of BTV are demarcated by red lines and are located broadly between the latitudes 35°S and 40°N (indicated by dashed lines). Other important *Culicoides* vectors are listed next to the continent in which they are involved in transmission. **b** | Map showing the distribution of BTV and the closely related orbivirus African horse sickness virus (AHSV) prior to 1998 (grey shading) and that of BTV since 1998 (up to November 2004, pink shading). Hatched lines indicate the approximate known northern and southern range limits of the three most important vector groups in Europe (derived from references cited in the text as well as REFS 136–143). Open circles indicate sites where *Culicoides imicola* was found to be absent before 1998.

source: Purse in NATURE 2005 "Climate change and the recent emergence of bluetongue in Europe"

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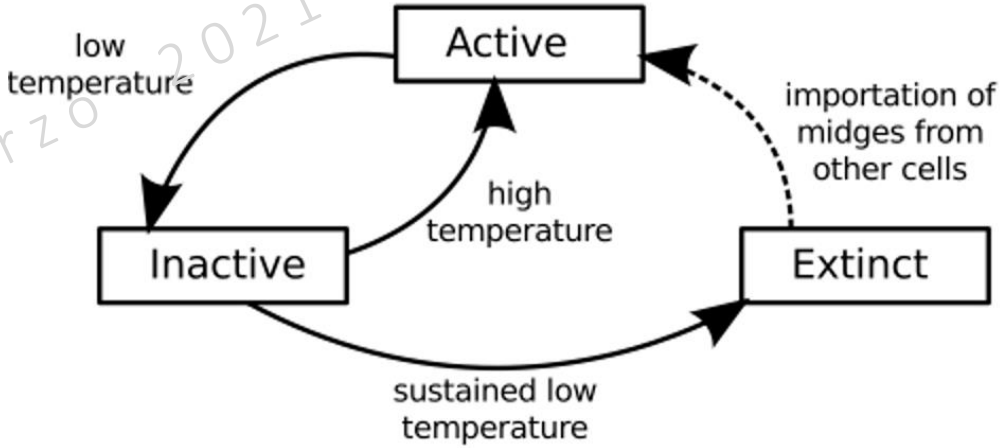
And in winter ?

- individuals surviving for up to three months at 10 °C in the laboratory
- *C. imicola* eggs survive 2 months <6°C
- *C. imicola* adults survive 2 weeks -1,5 °C
- *Adults <12°C not active*
- *Death only if* 0°C > 7 days
- most species of *Culicoides* at northern latitudes survive the winter as larvae



Kelso et al, 2014

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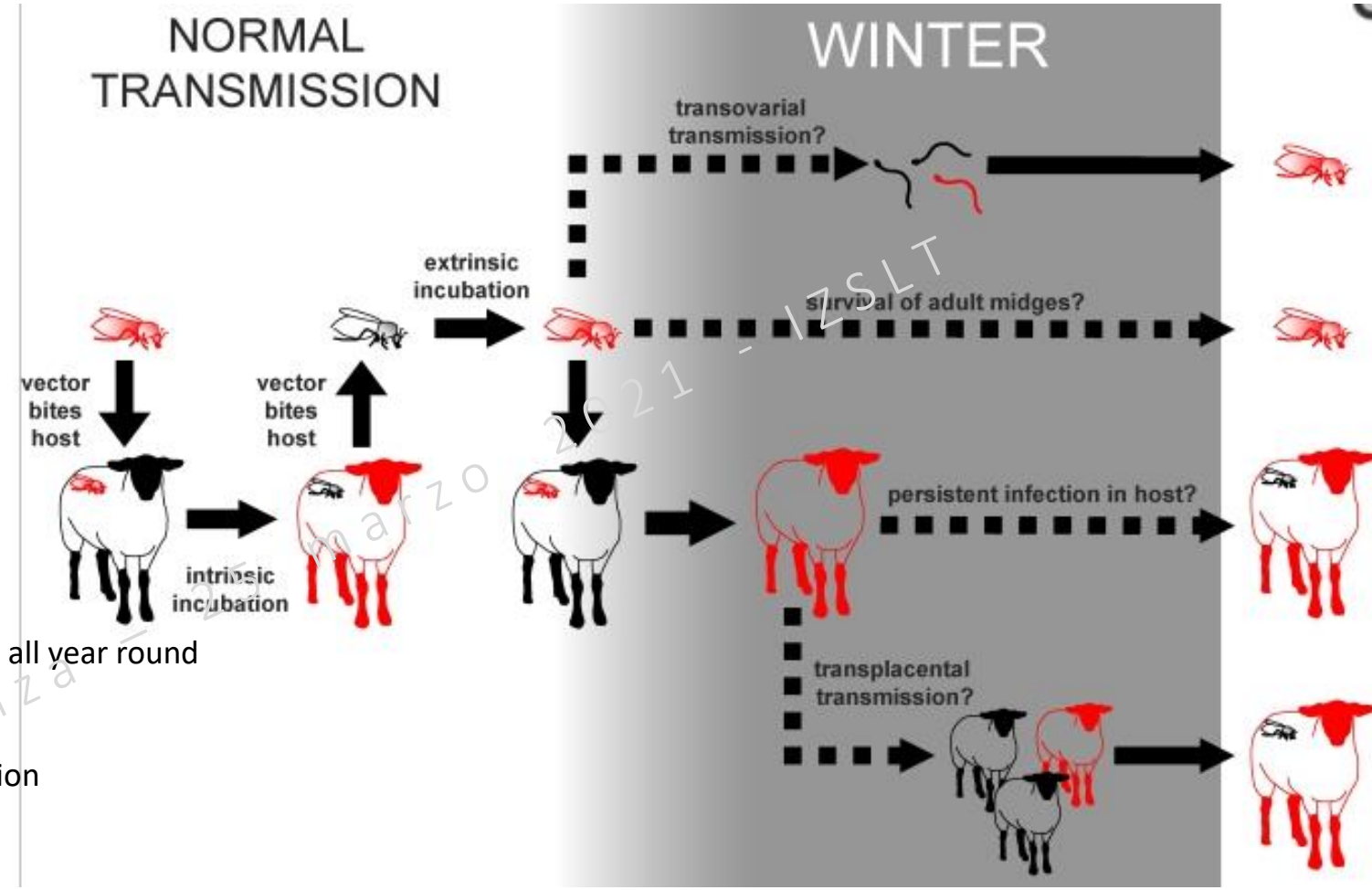

OVERWINTER IN VECTOR
vertical transmission
reported negative results
Inside buildings, conditions sufficiently warm for blood-feeding all year round

OVERWINTER IN RUMINANT
chronic or latent infection , transplacenta, horizontal transmission

OTHER
unknown reservoir
(white-tailed deer and elk)

Plos Biol. 2008 Aug; 6(8): e210.

oral transmission between mammalian hosts (consume placental material following a birth)

Carnivores can also become infected after ingesting infected meat and organs

BTV can also be spread mechanically between animals by humans, for example through contamination of vaccines

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Sfide per il futuro

- Virus continues to evolve
  - New genetic characteristics
  - Viral recombination exchanging genoma in
    - different virus in the same cell
    - Reassortant in progenie
- Epidemiology
  - Climate change
  - Anthropogenic drivers, notably role of ‘laboratory’ or live attenuated strains

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EU-Response to BT

- DATA
  - Siman, Wahis, ADNS, grey
  - Surveillance data
- LEGISLATION:
  - Council Directive 2000/75/EC
  - Commission Regulation (EC) No 1266/2007
  - AHL
  - Commission Decisions: Approval of programmes (monitoring, vaccination)
  - Opinions of EFSA
  - OIE Terrestrial Animal Health Code

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5. Normativa di riferimento

UE	Reg. (UE) 1266/2007 (consolidato) Dir. 2000/75	http://eur-lex.europa.eu/advanced-search-form.html?qid=1454427864783&action=update
Nazionale	Dispositivo dirigenziale 17522/2019 Nota Min. Sal. 24826/2020 Nota Min. Sal. 1215/2021	http://bluetongue.izs.it/pls/izs_bt/bt_gestmenu.bt_index https://www.izslt.it/sorveglianza-sanita-animale/approfondimenti/

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Definizione di CASO

OIE

- BTV **isolated** from a ruminant or camelid
- **antigen or RNA** in samples + **clinical** signs consistent with bluetongue, or + epidem linked to a suspected or confirmed case
- **antibodies** to structural or nonstructural proteins of BTV (not a consequence of vaccination + **clinical** signs consistent with bluetongue, or + epidem linked to a suspected or confirmed case

1266/2007

- **clinical** signs consistent with bluetongue
- **sentinel animal seroconverted**
- BT virus **isolated**
- animal positive to **serological tests**
- **antigen** or **RNA** specific identified

AND epidemiological data indicate that this is the result of virus circulation in the holding and not the result of the introduction of vaccinated *or seropositive* animals from RZ

17522

Caso SOSPETTO:

sintomo riferibile + dati **epidemiologici**

(sieroconversione sospetta non genera più restrizione)

Caso CONFERMATO:

- **sieroconversione** entro 60 gg dal prelievo precedente
- **clinica** anche da sola se epidemia
- **PCR**
- **isolamento** del virus

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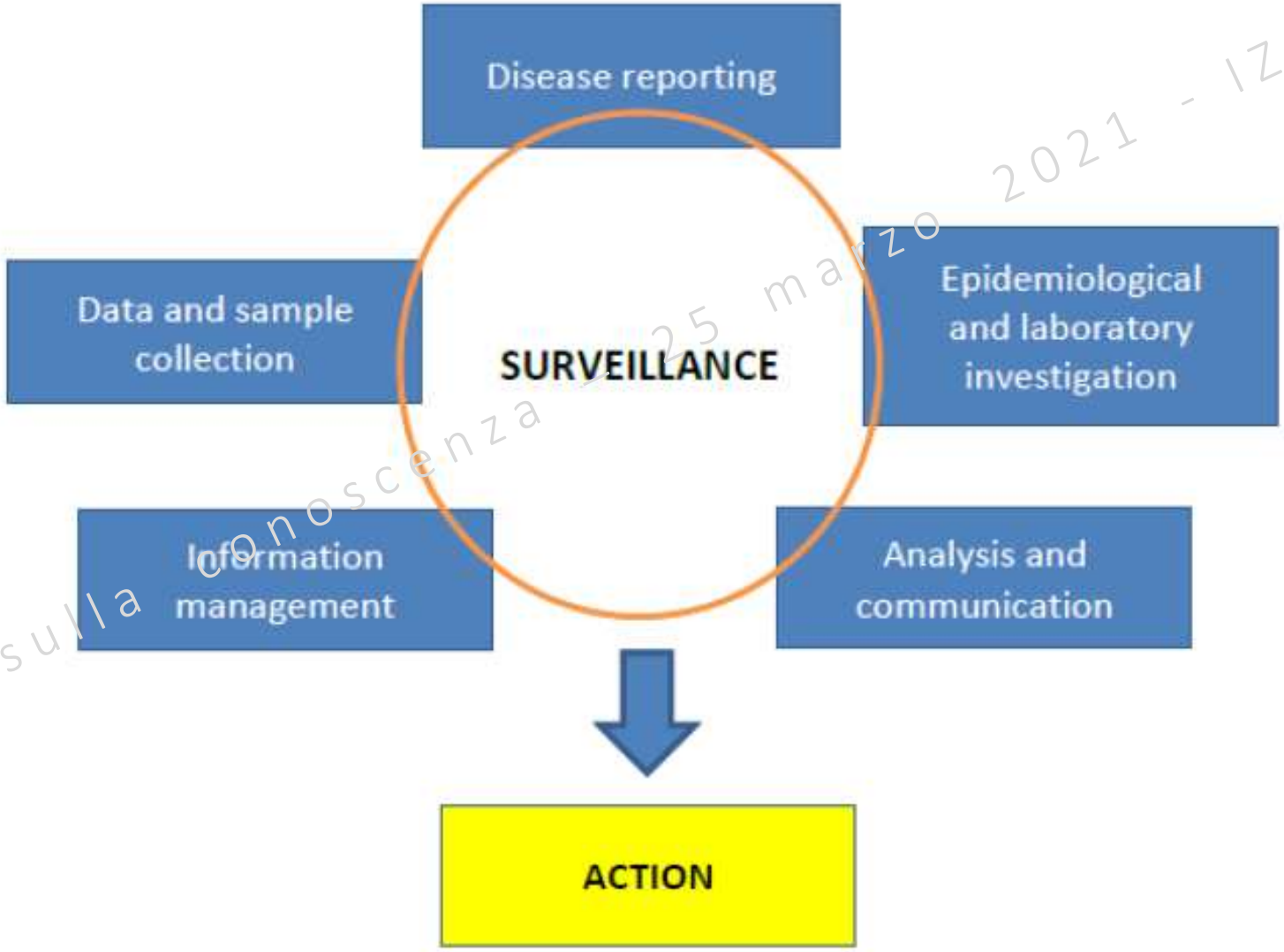
Surveillance



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SORVEGLIANZA DELLA BT

1. CLINICAL surveillance
2. SEROLOGICAL and VIROLOGICAL surveillance on domestic ruminants (mainly cattle)
3. ENTOMOLOGICAL surveillance
4. Supplementary measures: wildlife surveillance, etc

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SORVEGLIANZA DELLA BT

CLINICAL SURVEILLANCE

- Passiva (sensibilità max in early/introduced detection)
- should always be confirmed by laboratory testing
- Allevatore chiama la Asl?

SEROLOGICAL SURVEILLANCE

- one of the most effective methods of detecting BTV
- cattle are the most sensitive indicator species
- sentinels should be individually identified
- possible to use sera collected for other survey purposes (WND) or abattoir
- cross reaction in country where BT present for many years

- Antibodies against BTV. Positive test results can have four possible causes:
  - natural infection (how old ?)
  - vaccination
  - maternal antibodies

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Surveillance strategies SENTINEL animals

- preferred strategy for BTV surveillance for years (oggi sierotipo)
- groups of unexposed animals at fixed locations and sampled regularly
  [animals of known source and history of exposure]
- to avoid bias, sentinel groups should comprise animals selected to be of similar age and susceptibility to BTV infection
- frequency of sampling will depend on
  - ✓ if virus isolation is required, sentinels should be sampled at sufficiently frequent intervals to ensure that samples are collected during the period of viraemia (40-60 days)
  - ✓ Settaggio rilevazione precoce

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Surveillance strategies SENTINEL animals

- serum bank to allow retrospective studies
- necessary to differentiate newly infected animals from the seropositive animals due to past exposure to the virus.
- autochthonous animals (low turnover; reproduction better than fattening)
- once a sentinel has seroconverted, it should not be tested again new sentinels replacing such useless sentinels
- the adoption of restriction measures only on the infected holding might be a disincentive to the farmers to notify disease signs (funds)

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SEROLOGICAL SURVEILLANCE: tests to be used

- Basic test: serological test recommended for surveillance is the c-ELISA.
- ELISA shows highest sensitivity and reduced cost
- In addition, samples from the seroconverted animals may be tested with SN and/or PCR and/or other virological or serological methods to further confirm the serotype circulating in the zone

- Ex. France PCR

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PCR detect viraemia (40-60d) or infection tail (100d)

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Fig. 1. Application of laboratory tests in serological surveillance

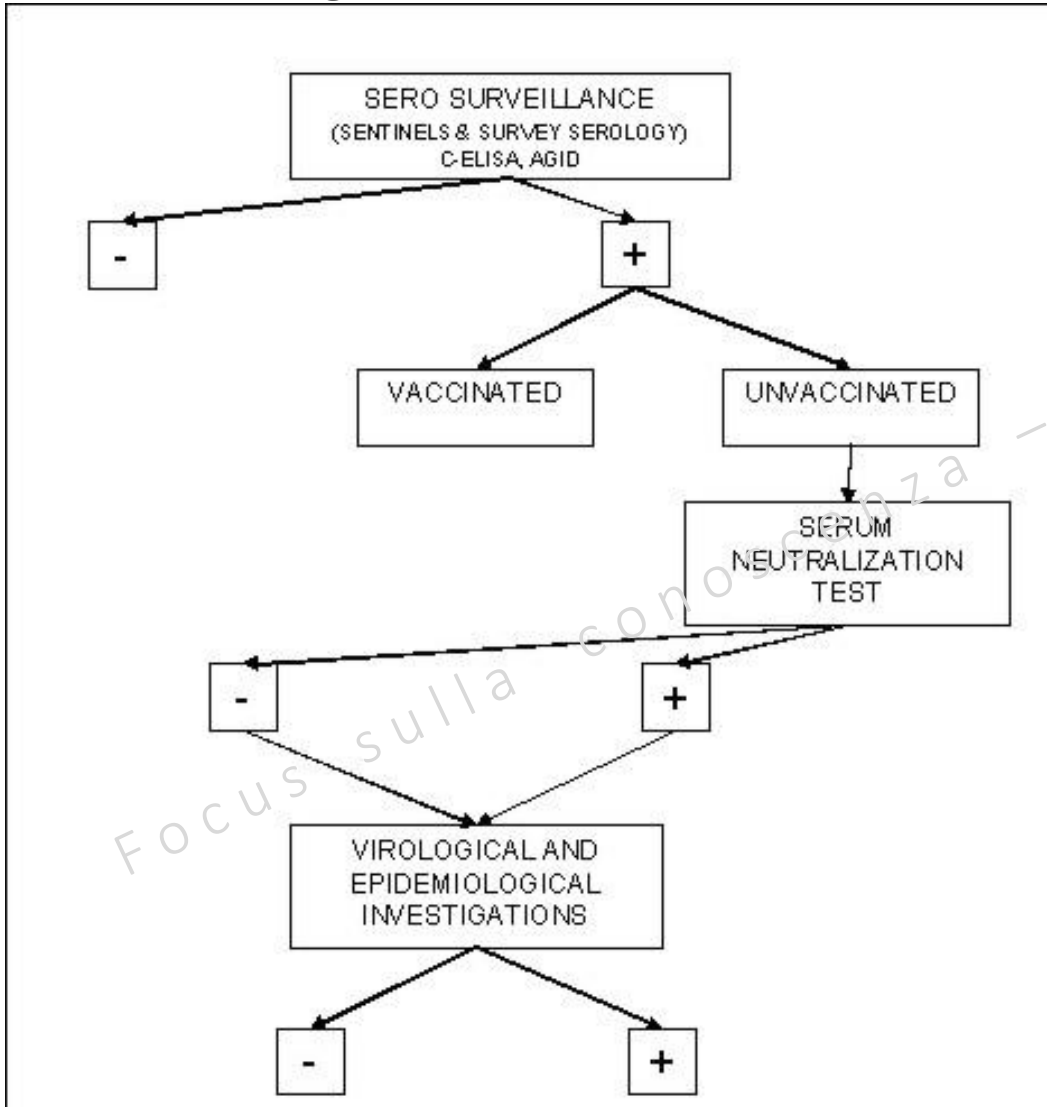
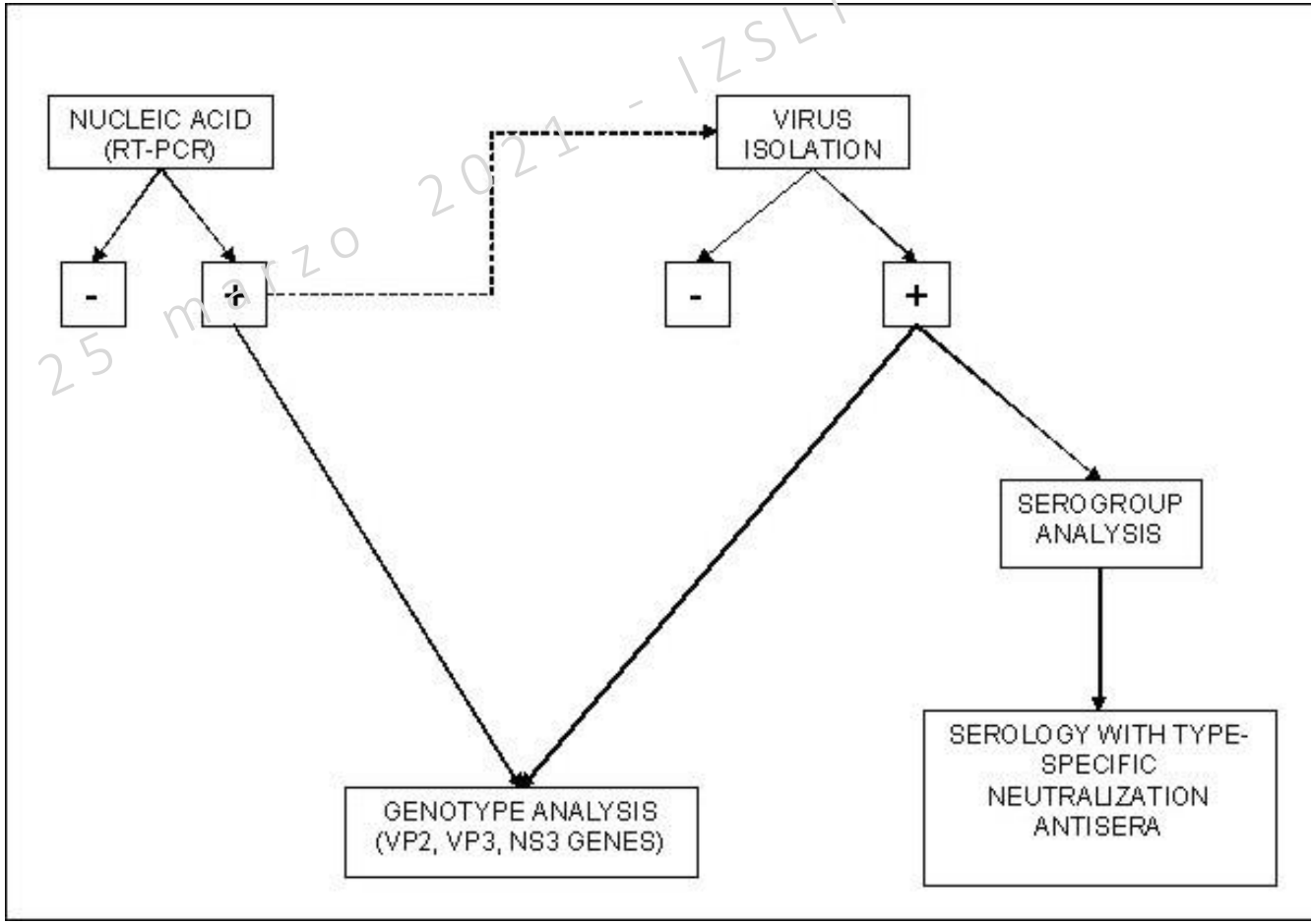


Fig. 2. Application of laboratory tests in virological surveillance



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SORVEGLIANZA DELLA BT

ENTOMOLOGICAL SURVEILLANCE

- permanent traps determine
  - population dynamics
  - abundance
  - over-wintering features (Seasonally free)

- non-permanent traps define
  - geographical distribution

- The use of a vector surveillance system to detect the presence of circulating virus is not recommended as a routine procedure as the typically low vector infection rates mean that such detections can be rare. NO as WND

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LA BT in ITALIA

- **risk assessment**

Scenarios

1. Control of disease with direct prophylaxis measures only;
2. Vaccination of sheep and goats in PZs;
3. Vaccination of all domestic ruminants (sheep, goats, cattle and buffaloes) in PZs

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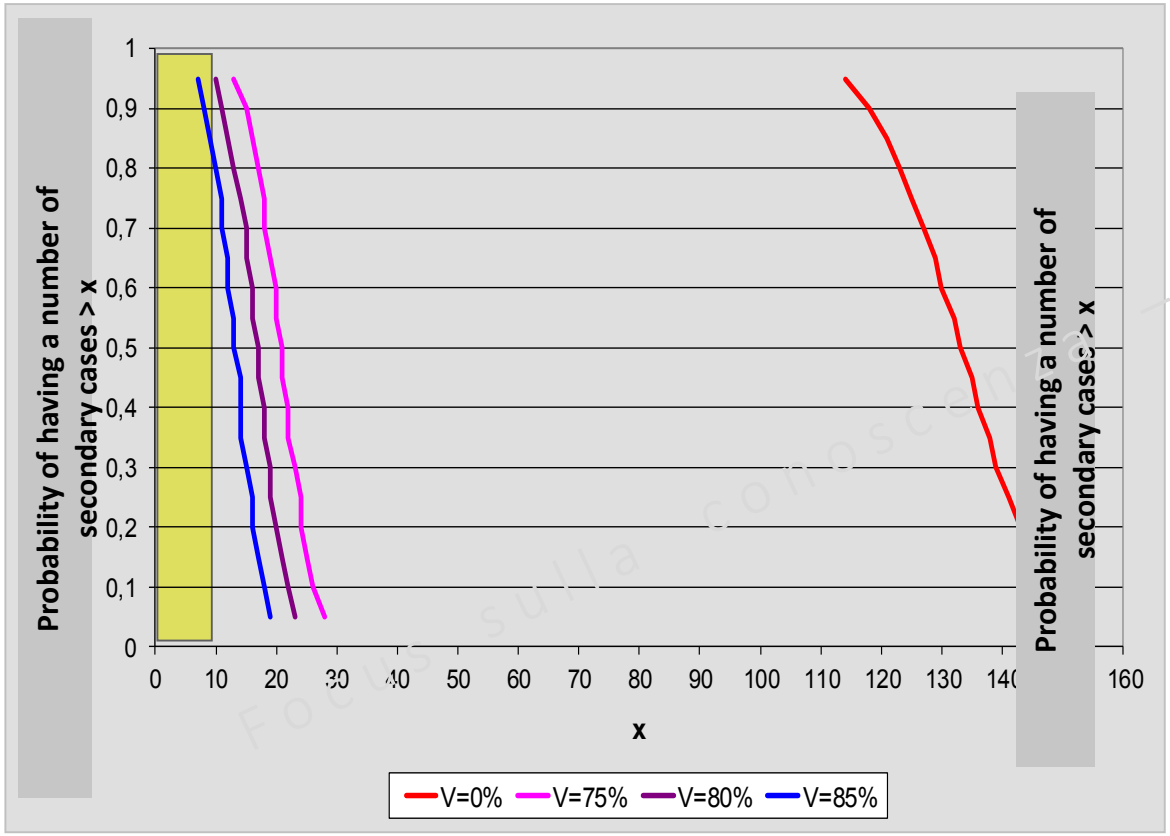
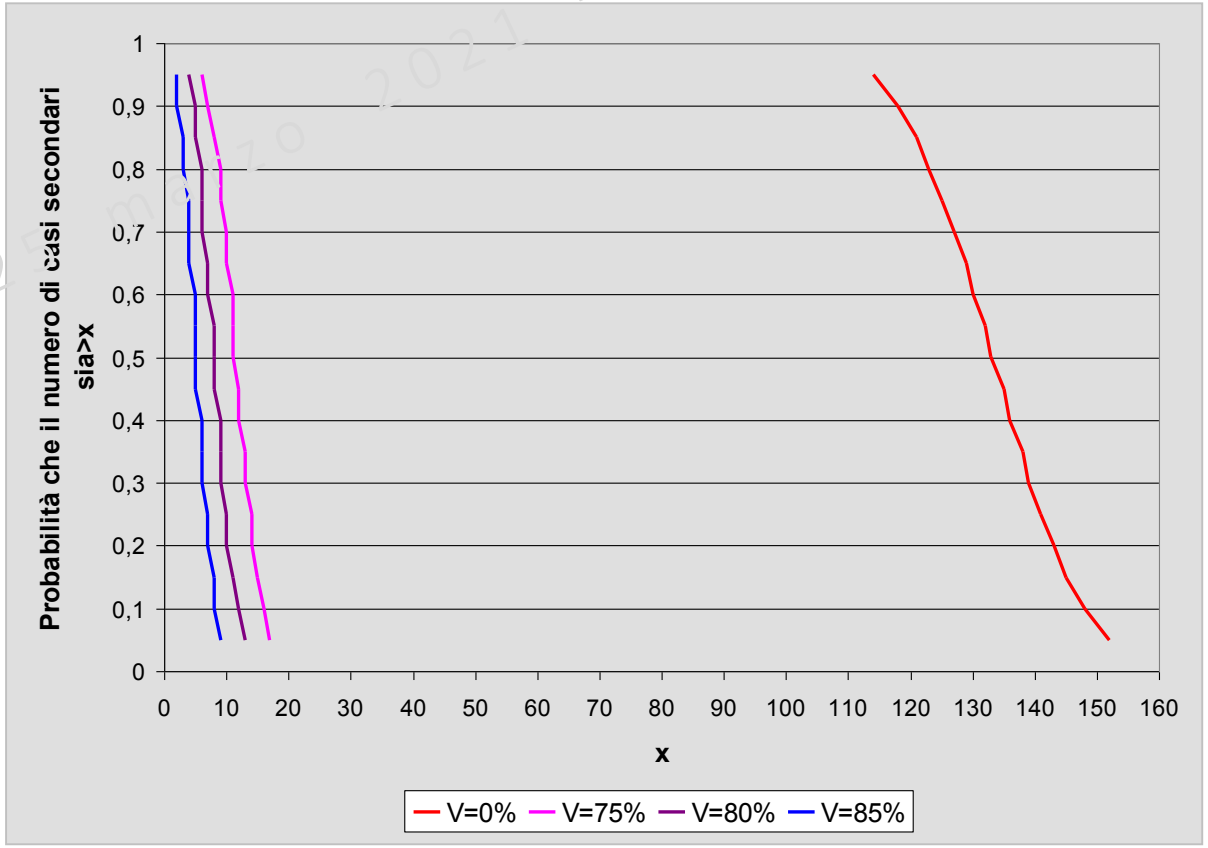
Vaccination of sheep and goats only: Expected number of secondary cases (# of primary cases=10)



Vaccination of all ruminants: Expected number of secondary cases (# of primary cases=10)



Source: IZSAM

SENATO DELLA REPUBBLICA

XIV LEGISLATURA

COMMISSIONE PARLAMENTARE D'INCHIESTA

SULL'EFFICACIA E L'EFFICIENZA DEL SERVIZIO SANITARIO NAZIONALE, NONCHÉ SULLE CAUSE DELL'INCENDIO SVILUPPATOSI TRA IL 15 E IL 16 DICEMBRE 2001 NEL COMUNE DI SAN GREGORIO MAGNO

INCHIESTA SULLE PROBLEMATICHE AFFERENTI IL CONTRASTO DELLA FEBBRE CATARRALE DEGLI OVINI (*BLUE TONGUE*)

11° Resoconto stenografico

SEDUTA DI MERCOLEDÌ 3 DICEMBRE 2003

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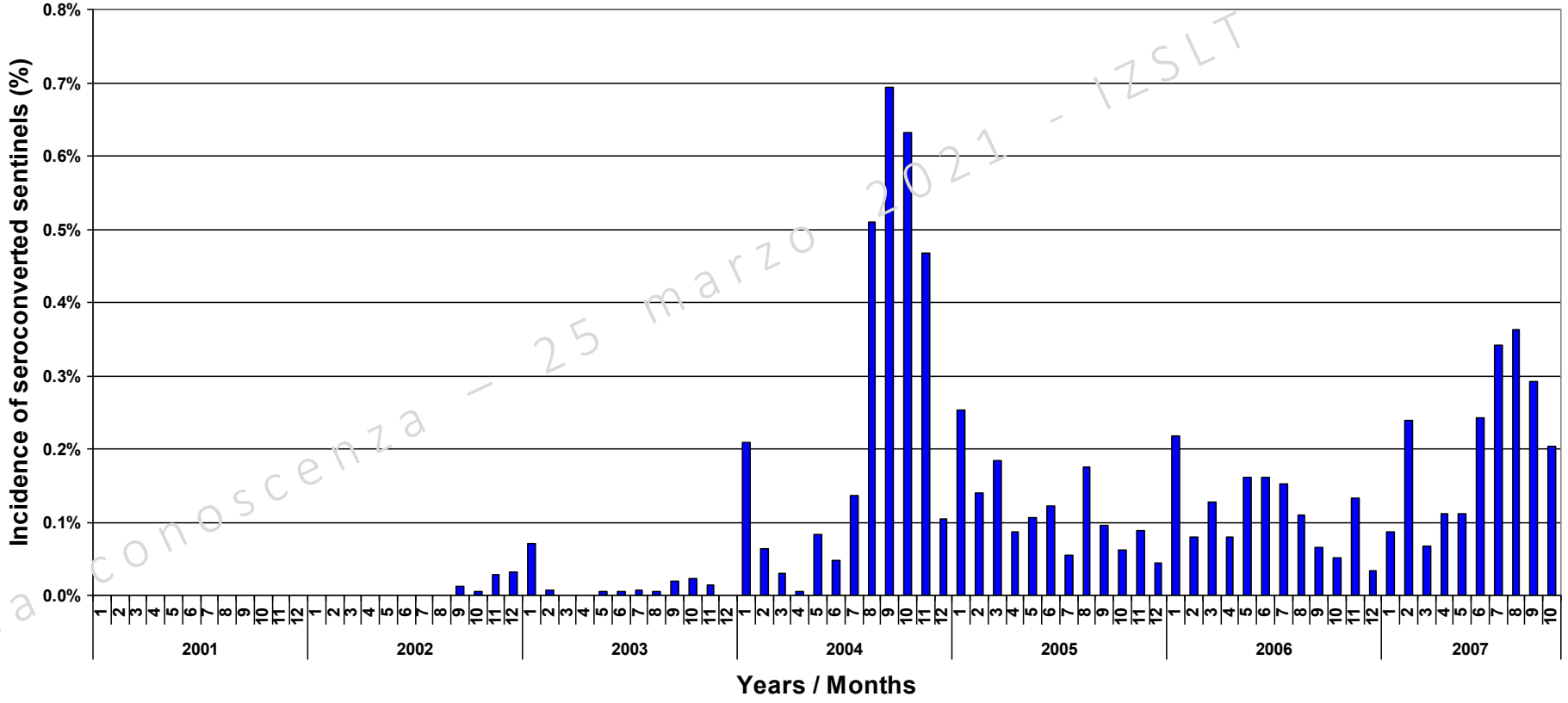
BTV 16



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Vaccination against BTV16 was discontinued in 2004

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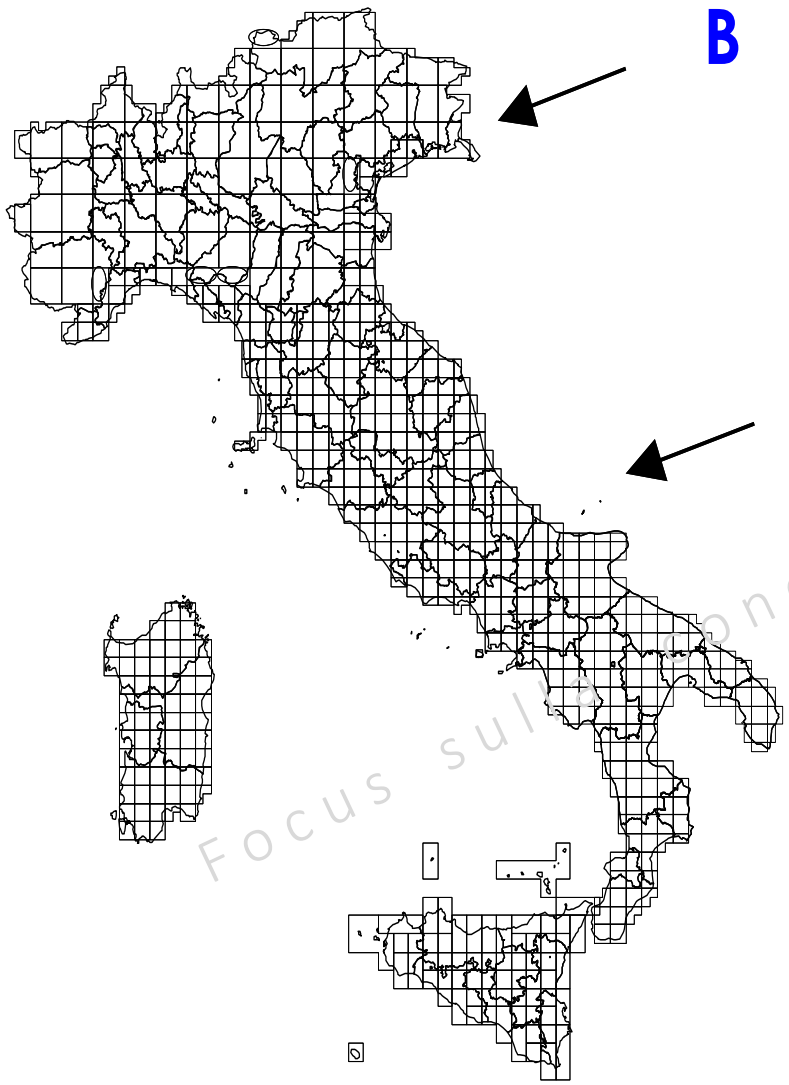
LA BT in ITALIA

Serological surveillance in Italy



- **B - LOW RISK**
  - **1600 km² each cell**
  - **148 sentinel animals (i.e. 185/2000 km²)**
  - **8-10 herds**
  - **Period**
    - **Every 60 dd (Jan-Apr)**
    - **Every 30 dd (May-Dec)**

- **A – HIGH RISK**
  - **400 km² each cell**
  - **58 sentinel animals (i.e. 290/2000 km²)**
  - **5-8 herds**
  - **Period**
    - **Every 30 dd (Jan-Apr)**
    - **Every 15 dd (May-Dec)**

Focus sulla conoscenza – 25 marzo 2021 - IZSLT

Surveillance strategies: Epidemiological unit

- If
- A farm is in a grid cell of 40x40 km
- all the sentinel animals in the cell react negative to the BT tests,
- given the sample size used

it means that

with 95% probability, during the previous month, in that cell the incidence of BT has been below the design incidence (derived from the affected areas of southern Europe) or possibly nil,

or, in other words

the nearest BTV circulation is more than 20 km apart

Focus sulla conoscenza - 25 marzo 2021 - IZSLT

Istituto Zooprofilattico Sperimentale del Lazio e della Toscana *M. Aleandri*

Serological surveillance in Italy

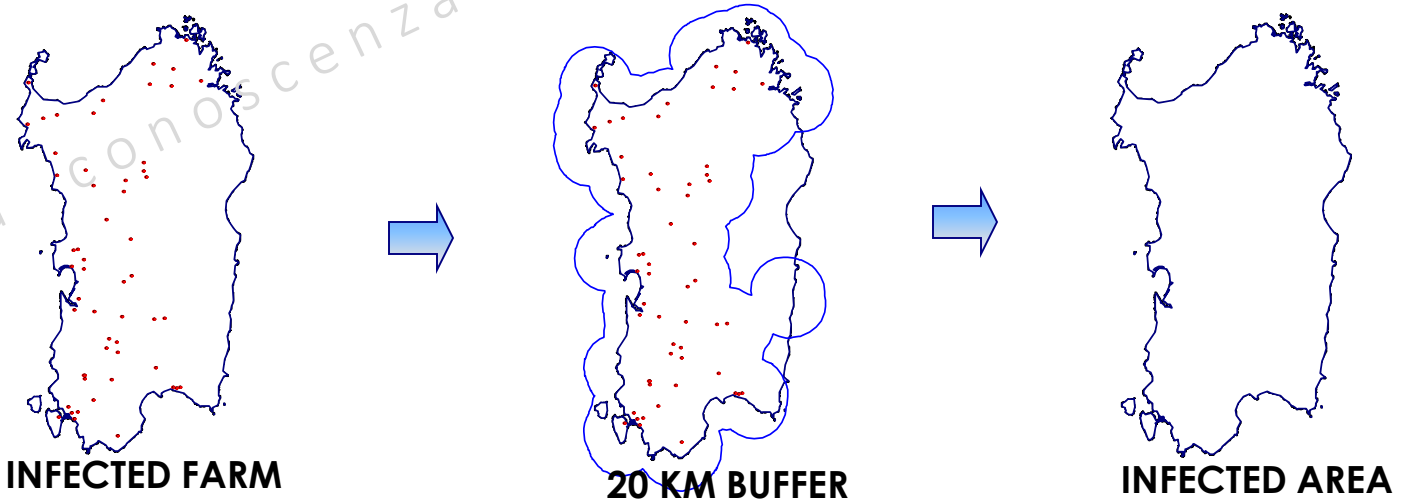
Focus sulla conoscenza – 2 marzo – IZSLT

After BTV8 epidemics, whole national teriitory was considered AREA A

Uso della sorveglianza per definire zone soggette a restrizioni

- ZONA INFETTA
  - Infected area:
    - all Municipalities within a buffer of 20 km of radius from an infected farm

Focus sulla conoscenza – 25 marzo 2021 - IZSLT



Istituto Zooprofilattico Sperimentale del Lazio e della Toscana *M. Aleandri*

Serological surveillance in Italy

- 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**

Focus sulla conoscenza – 25 marzo 2021 IZSLT

- **(*) For each sample tested by VN, the presence of antibodies against 8/9 serotypes is checked: BTV-1, BTV-2, BTV-3, BTV-4, BTV-6, BTV-8, BTV-9, BTV-14, BTV-15, BTV-16**

Source: IZSAM

Istituto Zooprofilattico Sperimentale del Lazio e della Toscana *M. Aleandri*

Entomological surveillance in Italy

- Location of permanent black-light traps
  - 1600 km^2 each cell
  - 1 trap and 1 catch per week in each cell
  - More than 250 black-light traps all over Italy (weekly)

Source: IZSAM

Focus sulla conoscenza – 25 marzo 2010 – IZSLT

Istituto Zooprofilattico Sperimentale del Lazio e della Toscana *M. Aleandri*

The 1st re-planning of bluetongue surveillance in Italy

- A Monte Carlo simulation model
- The aim was to simulate:
  - expected number of animals to seroconvert and to be detected upon subsequent testing
  - expected number of herds in which at least one animal seroconverts to BTV and for the animal to be detected upon subsequent testing

- The following two groups of scenarios were considered:

  - a decrease in the number of sentinel herds (sentinel herds = 100, 90, 80, 70, 60, 50, 40, 30, 20, 10), and a constant number of sentinel animals per herd (sentinel animals = 12)
  - a decrease in the number of sentinel animals per herd (sentinel animals = 12, 11, 10, 9, 8, 7, 6, 5, 4), and a constant number of sentinel herds (sentinel herds = 100)

Focus sulla conoscenza – 25 marzo 2021 - IZSLT

Results: decrease in the number of sentinel herds

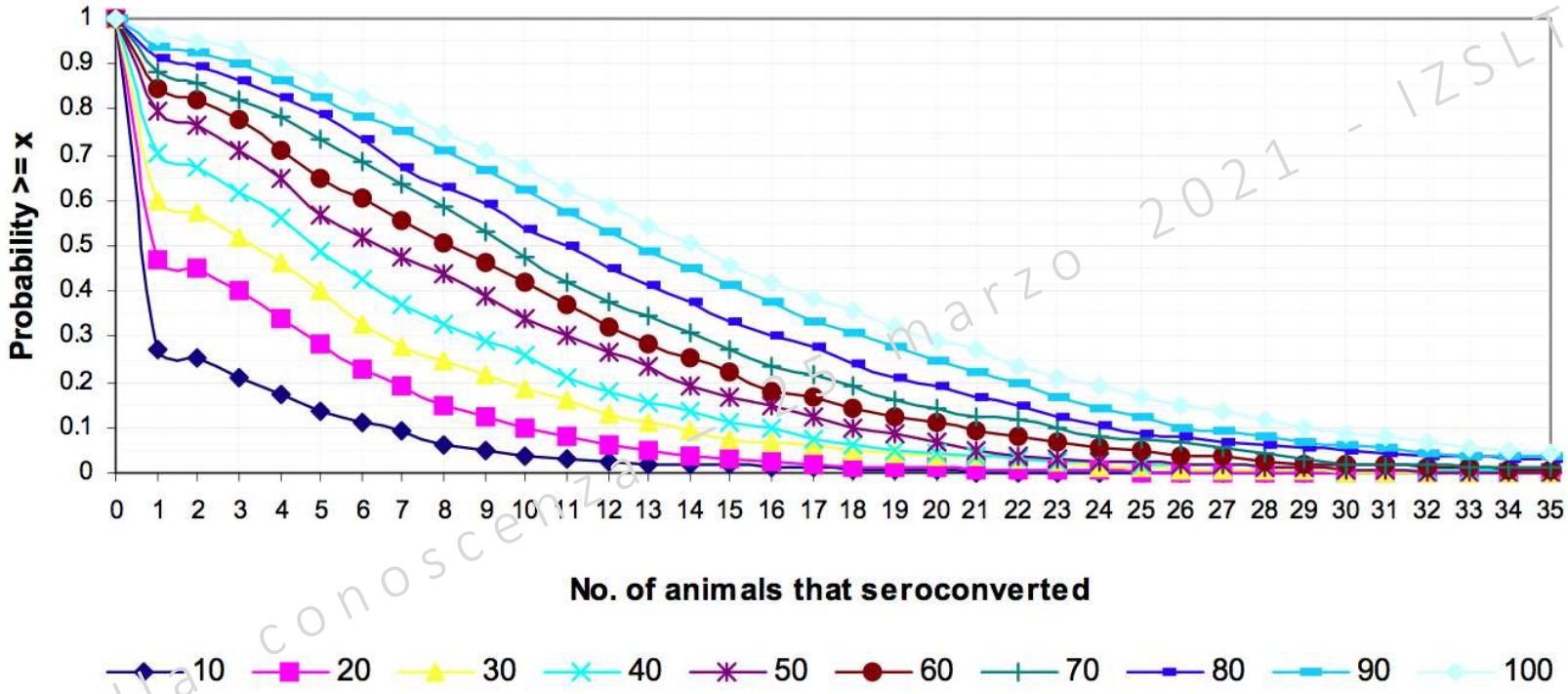


Figure 5
Model outcome: inverse cumulative distribution of probability of having one or more animals to seroconvert
Consideration is made of different scenarios of tested herds (10, 20 , 30 … 100) and the following variables:
5% incidence of infected herds
25% of incidence of infection within herds
12 sentinel animals tested for each herd

Source:
IZSAM

Focus sulla conoscenza 2 marzo 2021 - IZSLT

Results: decrease in the number of sentinel animals

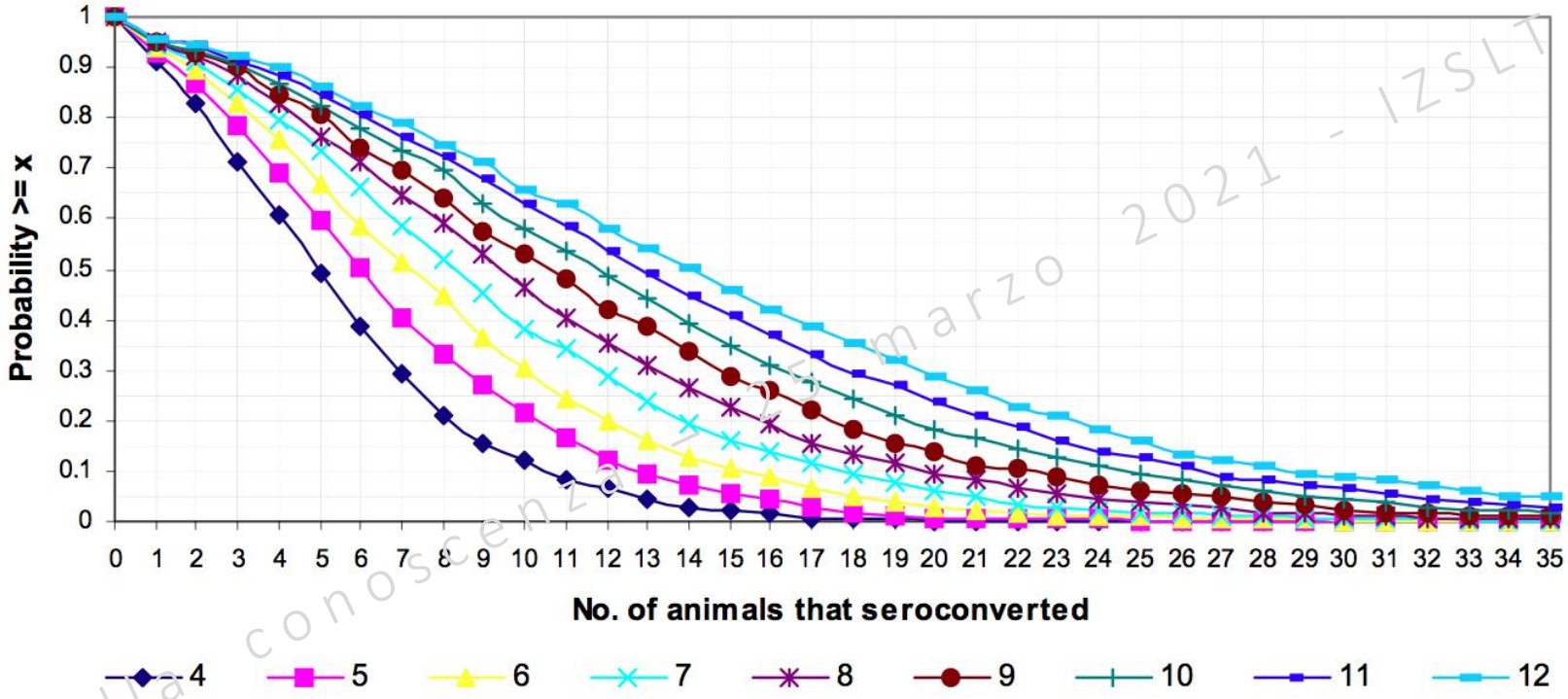


Figure 6
Model outcome: inverse cumulative distribution of probability of having one or more animals that seroconvert
Consideration is made of different scenarios of tested animal for each herd (4, 5, 6 … 12) and the following variables:
5% incidence of infected herds
25% incidence of infection within herds
100 sentinel herds tested

Source:
IZSAM

Focus sulla conoscenza - 2 marzo 2021 - IZSLT

Istituto Zooprofilattico Sperimentale del Lazio e della Toscana *M. Aleandri*

Vaccination in the EU in 2016

VACCINATION AGAINST BLUETONGUE						
COMPULSORY			VOLUNTARY			No program
Whole territory	Part territory (free zone)	Restricted zone	Whole territory	Part territory (free zone)	Restricted zone	
Bulgaria (BTV4), **Slovenia** (2017, BTV4), **Cyprus** (BTV8)	**Spain** (bovine, ovine BTV8)	**Croatia** (BTV4),**Portugal** (ovines BTV1, BTV4), **Spain** (bovine, ovine BTV1, BTV4)	**Austria** (BTV4 , BTV8), Netherlands (BTV4, BTV8), **Romania** (BTV4), **Slovak Republic** (BTV4), UK (BTV1, BTV2, BTV4, BTV8), Germany (BTV4, BTV8), Luxembourg (BTV8), Belgium (BTV 8)	**Spain** (bovine, ovine BTV8)	**Hungary** (BTV4), **Portugal** (bovines, caprine BTV1, BTV4), **Spain** (caprine BTV1, BTV4)	Czech Republic, Denmark, Finland, Latvia, Sweden, Lithuania, **Malta**, Poland, Estonia

Bluetongue: control, surveillance and safe movement of anir

Published: 8 March 2017 **Adopted:** 18 January 2017

EFSA Journal on the Wiley Online Library

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DOI: https://doi.org/10.2903/j.efsa.2017.4698

Metadata

EFSA Journal 2017;15(3):4698

Keywords: bluetongue, vaccination, surveillance, vector, Culicoides, insecticides

On request from: European Commission

Question Number: EFSA-Q-2016-00160

Animal Health and Welfare

Contact: alpha@efsa.europa.eu

Panel members at the time of adoption

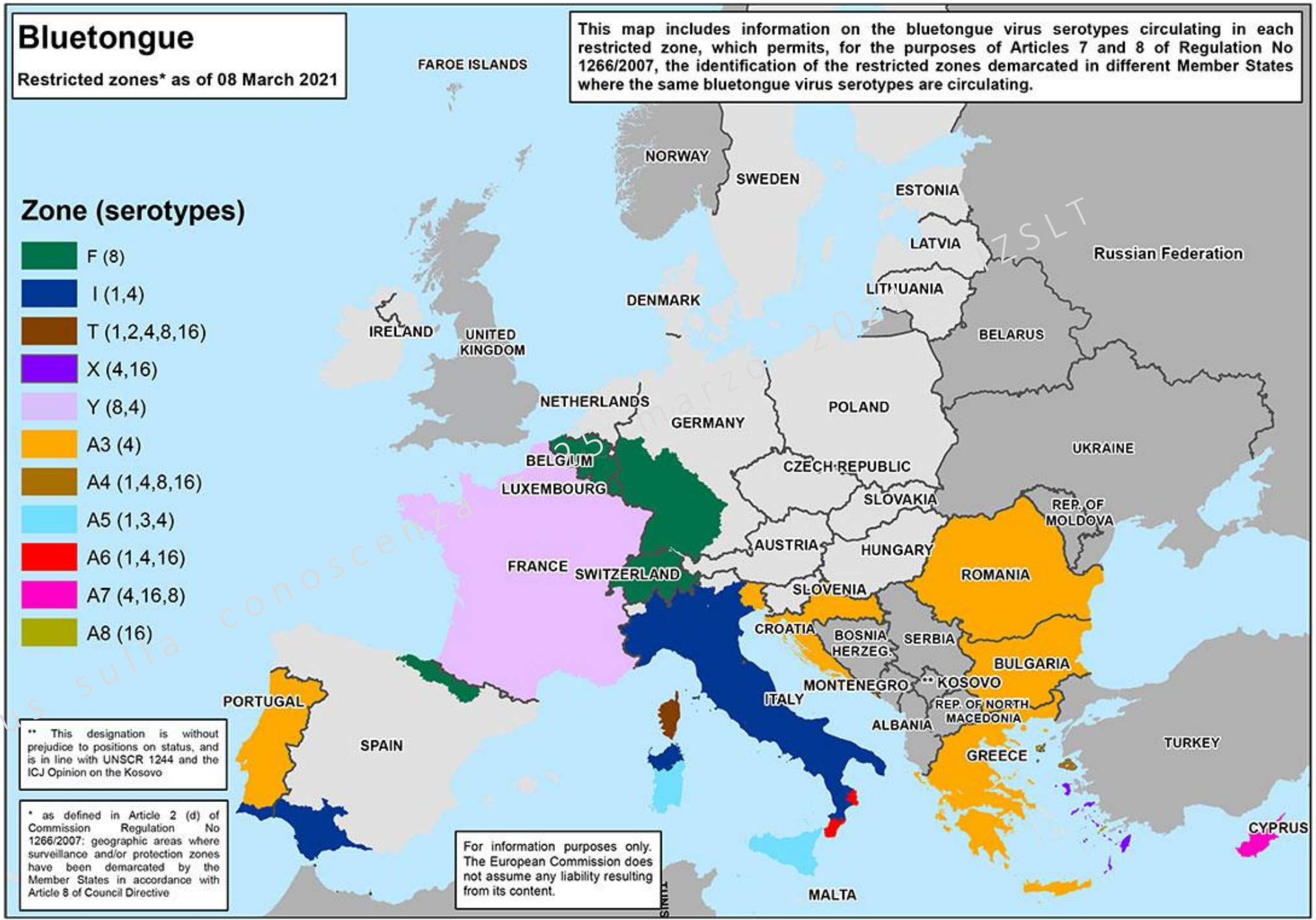
Miguel Angel Miranda, Jan Arend Stegeman, Dominique Bicout, Anette Botner, Andrew Butterworth, Paolo Calistri, Klaus Depner, Sandra Edwards, Bruno Garin-Bastuji, Margaret, Good, Christian Gortazar Schmidt, Virginie Michel, Simon More, Mohan Raj, Søren Saxmose Nielsen, Lisa Sihvonen, Hans Spoolder, Hans H. Thulke, Antonio Velarde, Preben Willeberg and Christoph Winckler.

Abstract

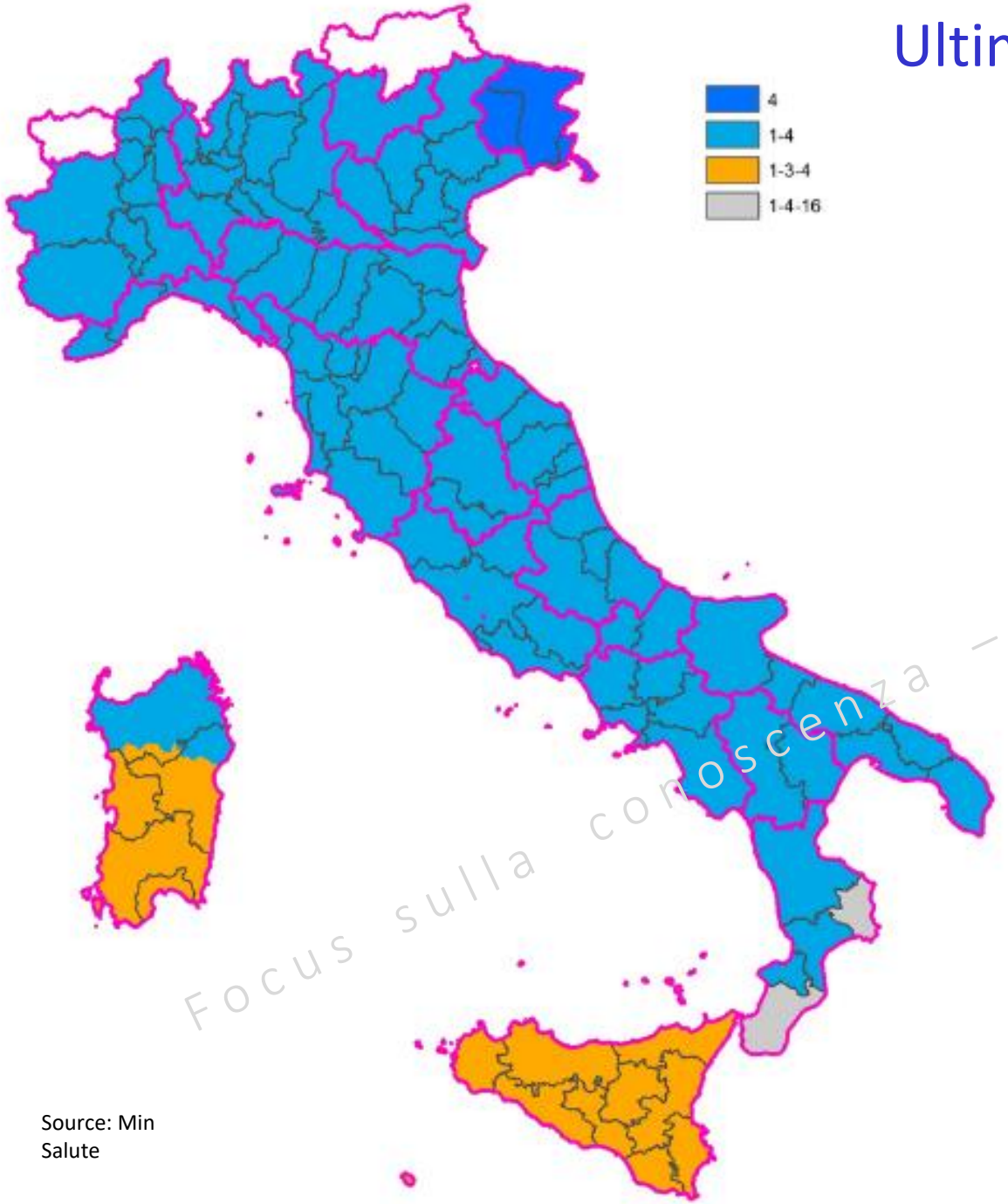
The performance of different bluetongue control measures related to both vaccination and protection from bluetongue virus (BTV) vectors was assessed. By means of a mathematical model, it was concluded that when vaccination is applied on 95% of animals even for 3 years, bluetongue cannot be eradicated and is able to re-emerge. Only after 5 years of vaccination, the infection may be close to the eradication levels. In the absence of vaccination, the disease can persist for several years, reaching an endemic condition with low

ıb/4698#panel-members-at-the-time-of-adoption ıms for bluetongue persistence, the persistence in the wildlife, the transplacental

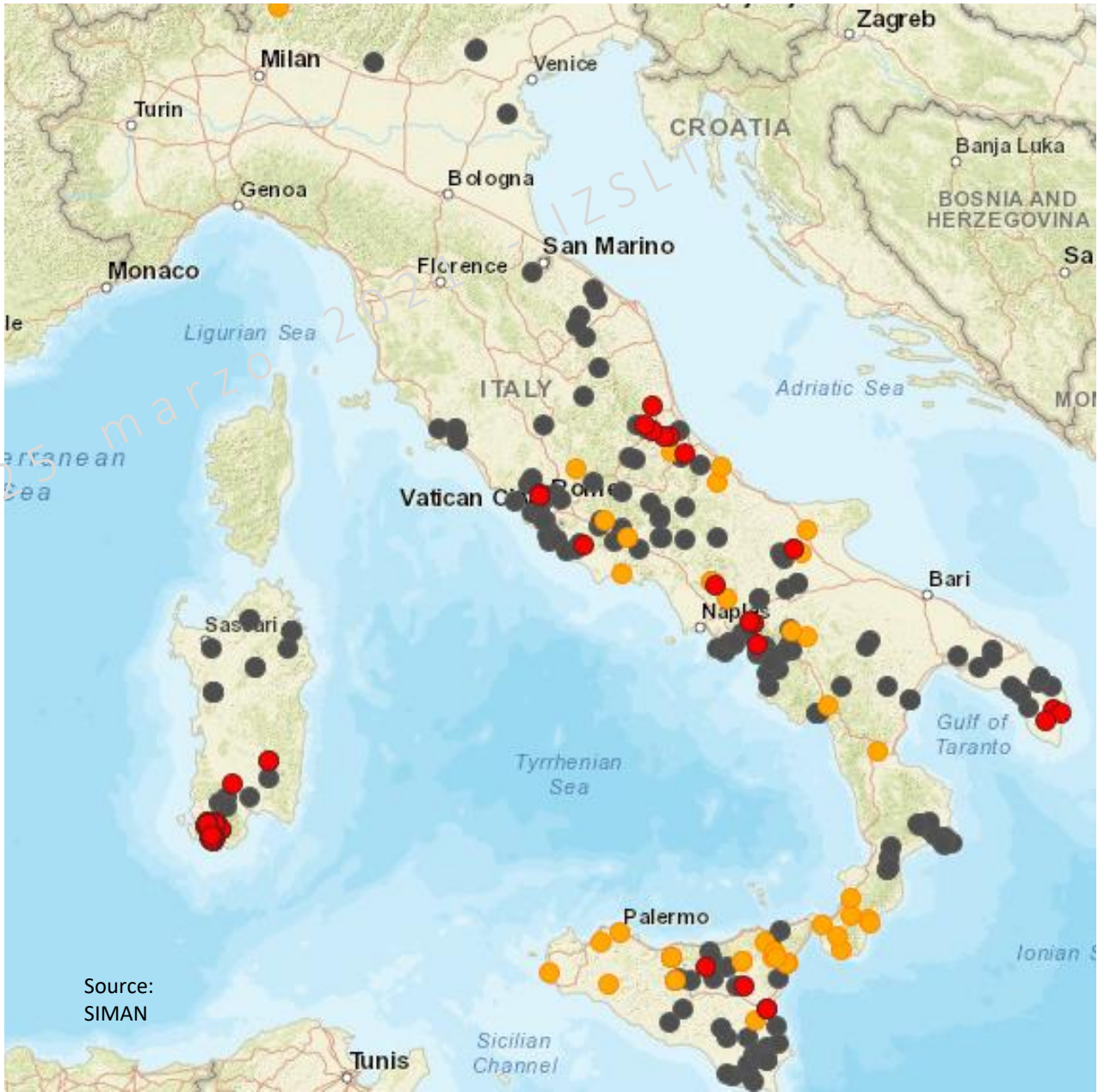
Focus sulla conoscenza
8 marzo 2021 - IZSLT


Bluetongue
Restricted zones* as of 08 March 2021
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.
Zone (serotypes)
F (8)
I (1,4)
T (1,2,4,8,16)
X (4,16)
Y (8,4)
A3 (4)
A4 (1,4,8,16)
A5 (1,3,4)
A6 (1,4,16)
A7 (4,16,8)
A8 (16)
FAROE ISLANDS
NORWAY
SWEDEN
ESTONIA
LATVIA
Russian Federation
LITHUANIA
DENMARK
IRELAND
UNITED KINGDOM
BELARUS
NETHERLANDS
GERMANY
POLAND
UKRAINE
BELGIUM
LUXEMBOURG
CZECH REPUBLIC
SLOVAKIA
REP. OF MOLDOVA
AUSTRIA
HUNGARY
FRANCE
SWITZERLAND
ROMANIA
SLOVENIA
CROATIA
BOSNIA HERZEG.
SERBIA
BULGARIA
MONTENEGRO
** KOSOVO
PORTUGAL
ITALY
REP. OF NORTH MACEDONIA
ALBANIA
SPAIN
GREECE
TURKEY
CYPRUS
MALTA
** This designation is without prejudice to positions on status, and is in line with UNSCR 1244 and the ICJ Opinion on the Kosovo
* 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
For information purposes only. The European Commission does not assume any liability resulting from its content.

Ultimi 2 anni: restrizioni vs circolazione virale



Source: Min Salute



Source: SIMAN

Focus sulla conoscenza – 25 marzo 2022 – IZSLT

Tabella 3. Focolai di bluetongue in Italia per anno e per sierotipo (fonte: Siman)

	2013	2014	2015	2016	2017	2018	2019	2020	Totale
BTV1	6776	1614	145	97	59	26	18	6	8741
BTV2	3	3					1		6
BTV3					1	30	1	2	34
BTV4	2	38	188	1026	2852	68	77	129	4380
BTV16	3		1						4
sconosciuto								6	6
Totale	6784	1655	334	1123	2912	124	97	143	13171

Grafico 1. Focolai di bluetongue in Italia per anno e per sierotipo (fonte: Siman)

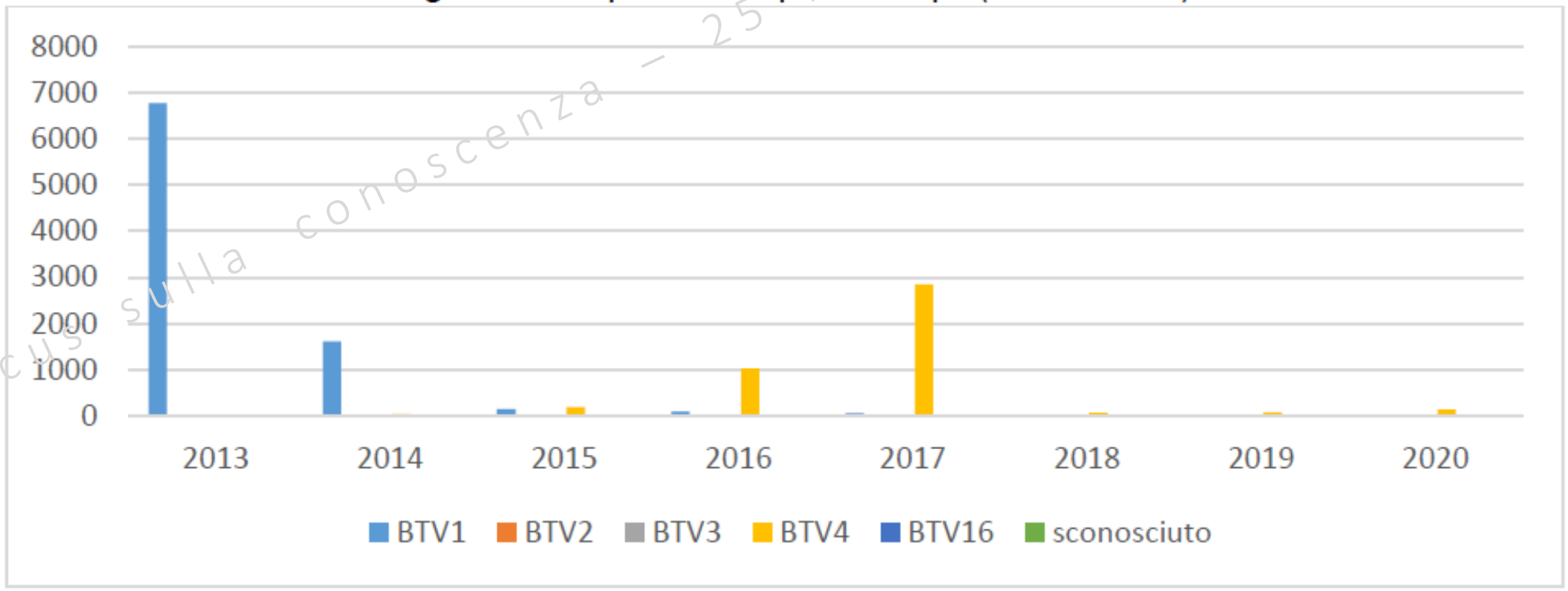


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Tabella 1. Focolai di bluetongue per sierotipo e macroregione nel 2019 (fonte: Siman)

Macroregione	BTV1	BTV2	BTV3	BTV4	Totale
Nord	1			3	4
Centro	3				3
Sud	12	1	1	65	79
Sardegna	2			9	11
Totale	18	1	1	77	97

Tabella 2. Focolai di bluetongue per sierotipo e macroregione nel 2020 (fonte: Siman)

Macroregione	BTV1	BTV3	BTV4	Sierotipo sconosciuto	Totale
Nord			1		1
Centro	2		59	3	64
Sud	2		63	3	68
Sardegna	2	2	6		10
Totale	6	2	129	6	143

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Nel corso del biennio 2019-2020, nella Regione Lazio, sono stati notificati in Siman 34 focolai di bluetongue (Tab. 4), l'82% dei quali causati da BTV 4. I focolai di tipo clinico sono stati circa il 20%, il 53% le positività diagnostiche e il 15% le sieroconversioni. Si segnala però un'errata attribuzione al tipo focolaio "positività diagnostica" di molti focolai ascrivibili al tipo "focolaio clinico", da cui è scaturita anche un'apposita nota (24286) del Ministero della Salute sulla definizione di focolaio.

Tutte le 5 province laziali rimangono in restrizione sia per il sierotipo 1 che per il sierotipo 4.

Le province dove si è riscontrata la maggior circolazione virale sono state quelle di Roma e Frosinone, nella provincia di Rieti è stato segnalato un solo focolaio. Nella provincia di Viterbo, a differenza delle precedenti epidemie, non è stata mai segnalata la circolazione del virus.

Focus sulla conoscenza _ 25 marzo 2021 IZSLT

Tabella 4. Dettaglio dei focolai di bluetongue nel 2019-2020 nel Lazio (fonte: Siman)

PROVINCIA	SIEROTIPO	TIPO FOCOLAIO				Totale
		FC	PD	SC	sconos.	
FR	BTV 1			1	1	2
	BTV 4		5	1		6
LT	sconos.				3	3
	BTV 4	1	3			4
RI	BTV 1			1		1
RM	BTV 4	6	10	2		18
Totale		7	18	5	4	34

FC: focolaio clinico; PD: positività diagnostica; SC: sieroconversione

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Grafico 3. Distribuzione temporale delle epidemie di Bluetongue 2013-2020 nel Lazio (fonte: Siman)

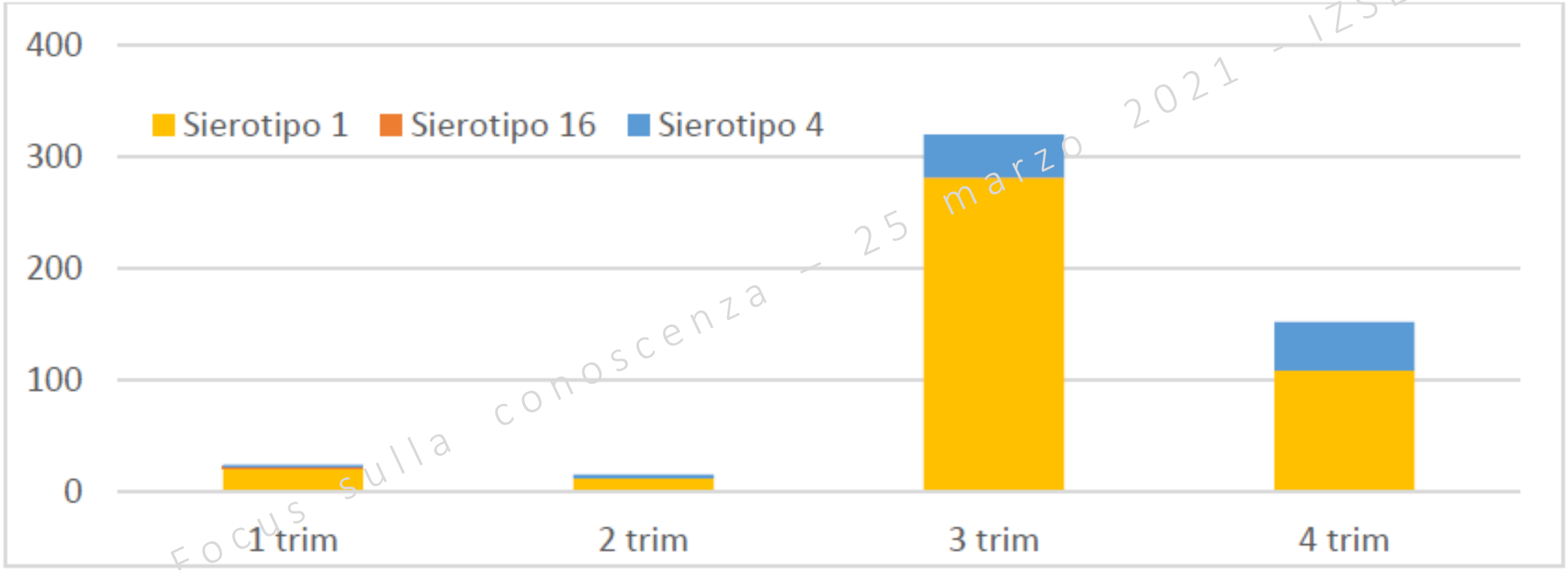

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Grafico 4. Attività di sorveglianza sierologica in provincia di Frosinone nel 2020 (fonte: OEVR)

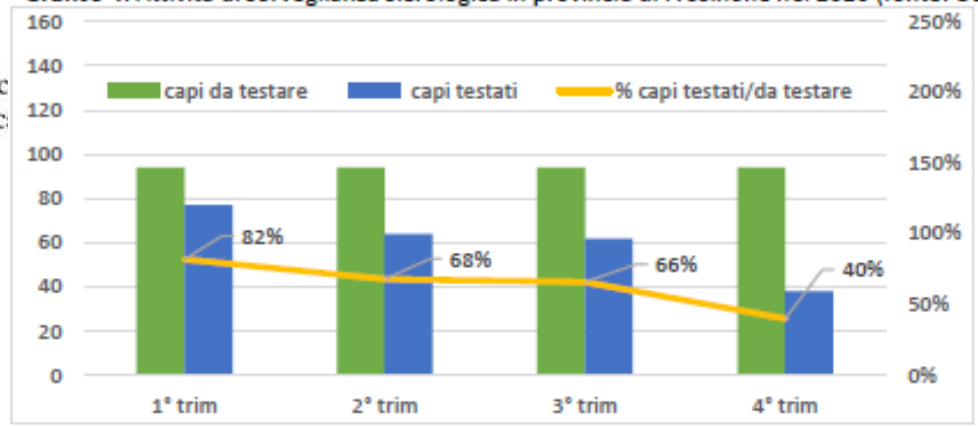

Grafico 5. Attività di sorveglianza sierologica trimestrale in provincia di Latina nel 2020 (fonte: OEVR)

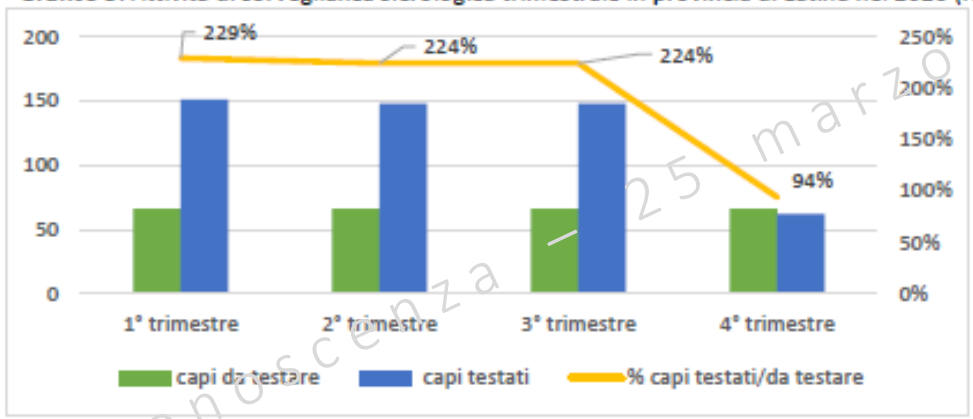

Grafico 6. Attività di sorveglianza sierologica trimestrale in provincia di Rieti nel 2020 (fonte: OEVR)

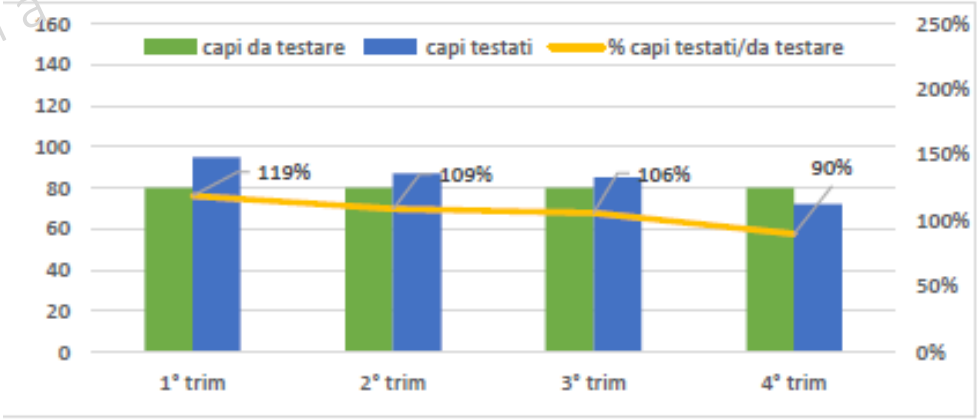

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Grafico 7. Attività di sorveglianza sierologica trimestrale in provincia di Roma nel 2020 (fonte: OEVR)

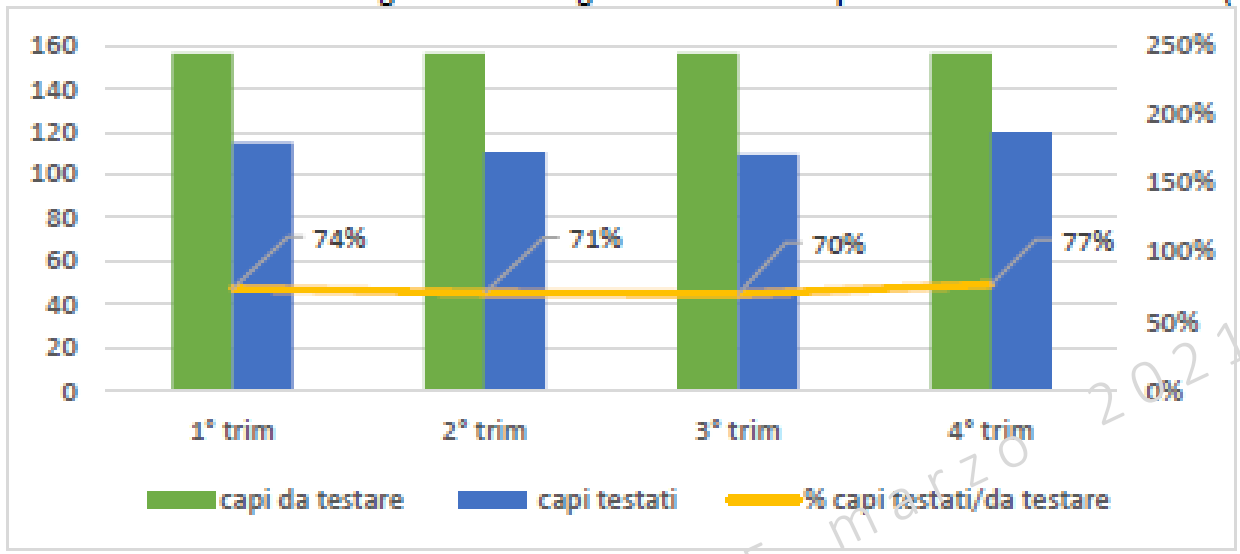

Grafico 8. Attività di sorveglianza sierologica trimestrale in provincia di Viterbo nel 2020 (fonte: OEVR)

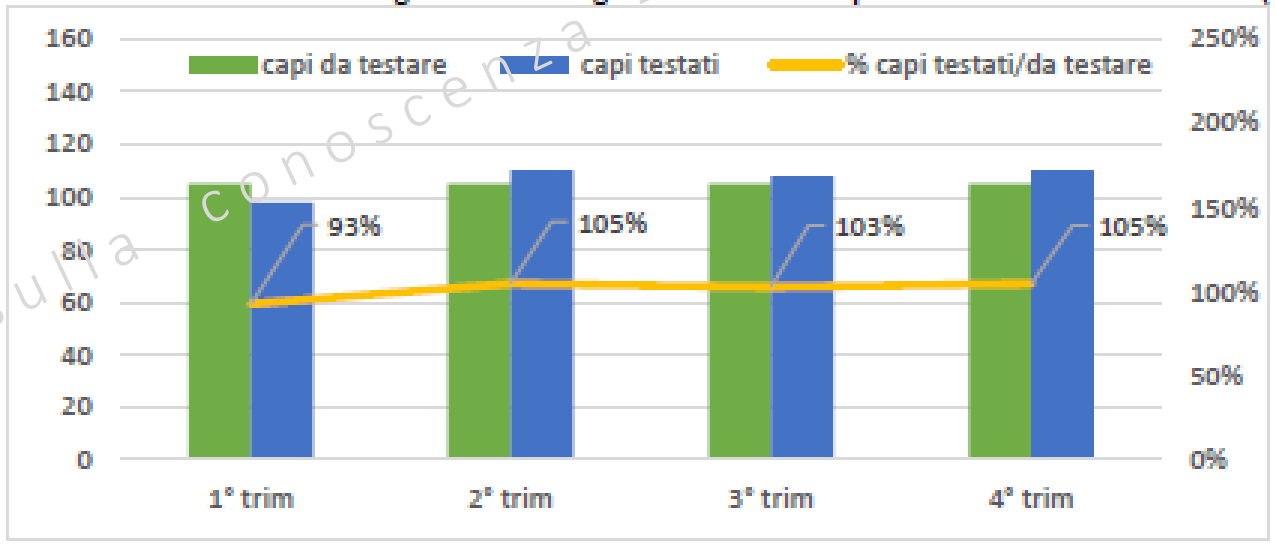

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Tabella 5. Attività di sorveglianza entomologica per BT nel Lazio nel 2019-2020 (fonte: OEVR)

Provincia	ASL	Numero catture (obiettivo=52) 2019	Numero catture (obiettivo=52) 2020
FR	FR	11	13
RI	RI	52	34
RM	RM 6	24	27
LT	LT	0	0
VT	VT	0	0

Focus sulla Conoscenza _25 marzo 2021 IZSLT

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MOVIMENTAZIONI DA MACELLO

Riferimenti normativi: Nota MinSal 17522/2019

DA	A MACELLO SITO IN	CONDIZIONI		MOVIMENTAZIONE
ovunque	ovunque	vaccinati o figli di madri vaccinate < 90gg		CONSENTITA (MinSal)
ZONA RESTRIZIONE	ZR = SIEROTIPI	tutti (anche non vaccinati)	• nessuna, anche impianto non designato	
ZONA RESTRIZIONE	Zona INDENNE o ZR ≠ SIEROTIPI	tutti (anche non vaccinati)	• Macello 1266 All IV • Trasporto senza sosta* • vincolo sanitario proc canal. • notifica tra ASL almeno 48h prima • Macellati entro 24 h (+ BDN 3 gg)	

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MOVIMENTAZIONI DA VITA

Riferimenti normativi: Nota MinSal 17522/2019

DA	A	ANIMALI	CONDIZIONI	MOVIMENTAZIONE
ovunque	ovunque	vaccinati o figli di madri vaccinate < 90gg		CONSENTITA (MinSal)
ZONA RESTRIZIONE	ZR = SIEROTIPI	tutti	• no segni clinici giorno trasporto	
		Ovini in azienda in buffer 20 km da caso confermato	• no segni clinici 24h prima Vet Ufficiale (riportato su Mod 4)	
	Stabilimenti a prova di vettore in Zona INDENNE o ZR ≠ SIEROTIPI	Non vaccinati < 90 gg	• az origine no buffer 20 km caso confermato • trasporto ore diurne (8-18); • insetto repellenti + insetticida • visita clinica ovini 24- 48 ore prima • vincolo sanitario + notifica almeno 48 ore • permanenza destino almeno 7 giorni. • test PCR individuale entro 24-36 ore dall'arrivo • divieto di movimentazione verso UE.	
	Zona INDENNE o ZR ≠ SIEROTIPI	non vaccinati	• PCR negativa 7 gg prima (a campione, max 57) • trasporto ore diurne (8-18) • insettorepellenti an.li 7 gg prima test, fino a trasporto compiuto • insetticidi mezzo attestati • vincolo sanitario • notifica almeno 48 ore	
		vaccinati	• > 90 gg vaccinati (anche 30 gg dopo prima dose)	
			• < 90 gg figli di madri vaccinate	
ZONA RESTRIZIONE	PSL Zona INDENNE o ZR ≠ SIEROTIPI	Non vaccinati	• TSL origine e/o destino per almeno 60 gg	

Focus sulla conoscenza – 25 marzo 2021 - IZSLT

Istituto Zooprofilattico Sperimentale del Lazio e della Toscana *M. Aleandri*

La Ricerca dell'IZSLT: after natural BT epidemics are sheep protected ?

- 2001 BTV2 epidemics
- 2002-2006 BTV-16 and BTV2 sporadic
- 2013/2014 epidemics 352 outbreaks (77% Cl Ou, 23% SC) (83% ovine)
- BTV1
- in 151 Municipalities . Almost 95% holding infected or buffer 4km

Focus sulla conoscenza – 25 marzo 2021 - IZSLT

ASL	SPECIE	TIPO FOCOLAIO	NUM CASI SIMAN	NUM MORTI SIMAN	NUM Ani PRESENTI	MORBILITA	MORTALITA	LETALITA
A.S.L. FROSINONE	OVINO	Focolaio Clinico	494	344	7661	**6,45**	**4,49**	**69,64**
A.S.L. LATINA			60	32	1514	**3,96**	**2,11**	**53,33**
A.S.L. RIETI			363	177	9916	**3,66**	**1,78**	**48,76**
A.S.L. 5 ROMA			26	16	2860	**0,91**	**0,56**	**61,54**
A.S.L. 6 ROMA			14	2	937	**1,49**	**0,21**	**14,29**
A.S.L. 7 ROMA/G			445	262	10755	**4,14**	**2,44**	**58,88**
A.S.L. 8 ROMA/H			90	88	853	**10,55**	**10,32**	**97,78**
A.S.L. VITERBO			594	273	11864	**5,01**	**2,30**	**45,96**
Totale			**2086**	**1194**	**46360**	**4,50**	**2,58**	**57,24**

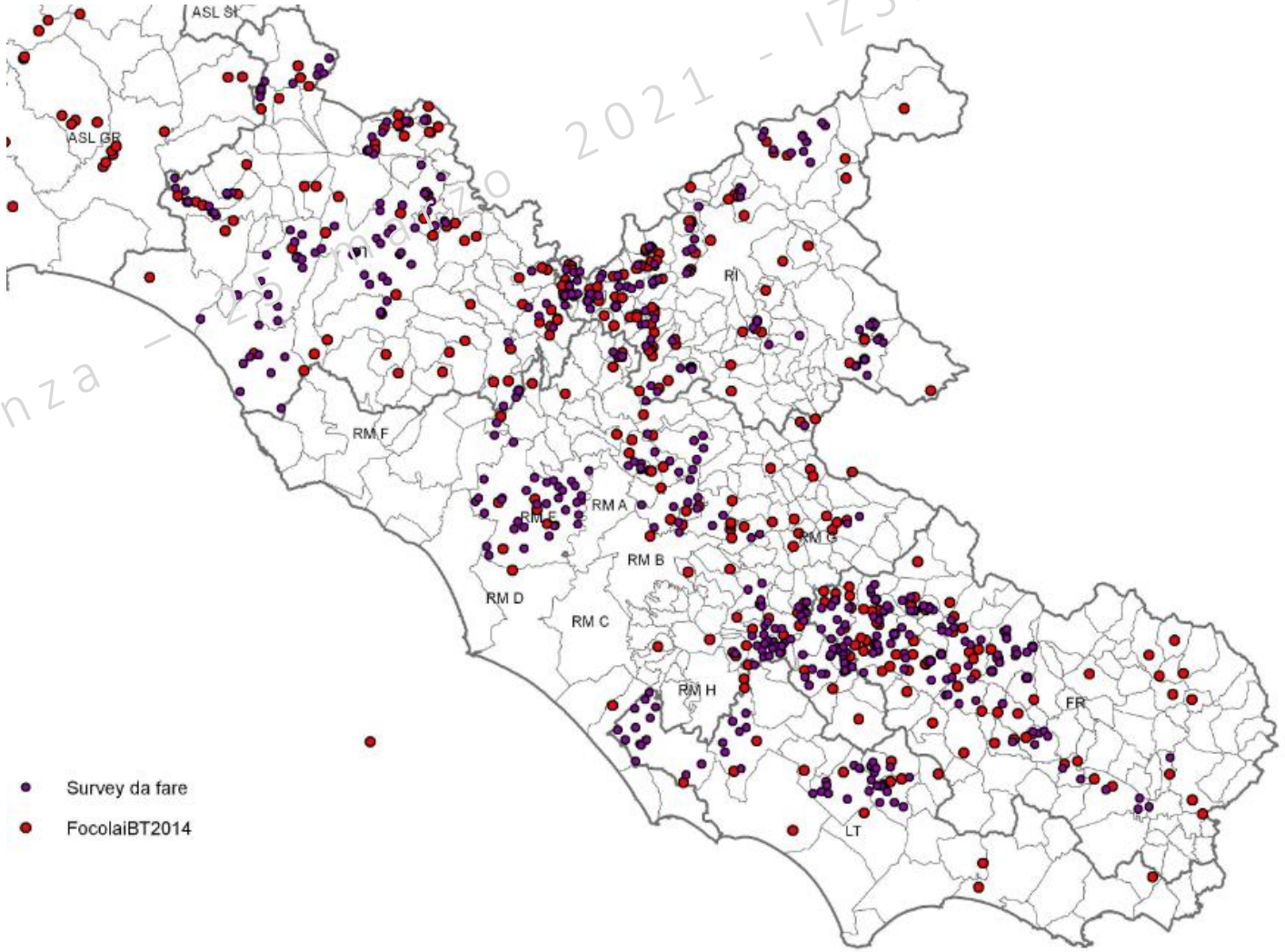
SURVEY 1.0 Around Outbreak

- *Kind of study: cross-sectional*
- *Area target: municipalities with at least 2 confirmed outbreak (77 with 285)*
- *sub-areas target stratified by LHU*
- Design of study:
  - Denominator ovine and caprine in BDN
  - C-Elisa
  - Exp P= 60%
  - SE= 5%
  - CL= 95%
- Stratified sample by No heads per Municipalities
- *No tested head per holdings*= 4/6
  - Number to reveal at least 1 positive if seroprev intraherd>= 0.60
  - Simple Random holdings selection.
  - No infected holdings
  - No vaccination holdings

Focus sulla conoscenza – 25 marzo 2021 - IZSLT

SURVEY 2.0 – In Outbreak

- *Kind of study: cross-sectional*
- *Area target: holdings ovine confirmed outbreak*
- Design of study:
  - 40 holdings * 30 Animals
  - C-Elisa
  - Exp P= 60%
  - SE= 5%
  - CL= 95%
- Stratified sample by LHU outbreaks
  - Simple Random holdings selection
  - No vaccination holdings



Focus sulla conoscenza – 25 maggio 2021 - IZSLT

Istituto Zooprofilattico Sperimentale del Lazio e della Toscana *M. Aleandri*

IZSLT SURVEY RESULTS

ASL	N campioni da fare	N campioni fatti	% positivita
RM F	24	24	**29,2**
RM H	6	6	**33,3**
RM G	344	329	**13,7**
RI	372	264	**33,3**
VT	372	274	**2,9**
FR	368	232	**32,3**
LT	318	138	**32,6**
RM E	222	111	
Totale	2026	1378	**17,1**

%	NF	F
0	211	3
0-30	87	24
31-60	61	8
61-80	18	3
81-100	17	0
Holdings	394	38
Animals	1764	1106

Focus sulla conoscenza – 25 marzo 2021 - IZSLT

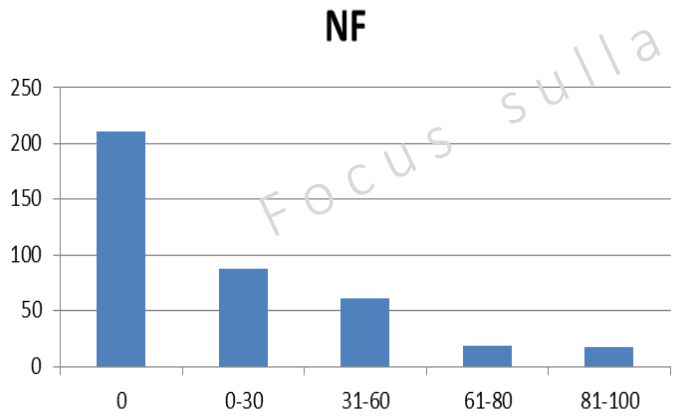
PLoS One. 2019 Jan 9;14(1):e0208074. doi: 10.1371/journal.pone.0208074. eCollection 2019.

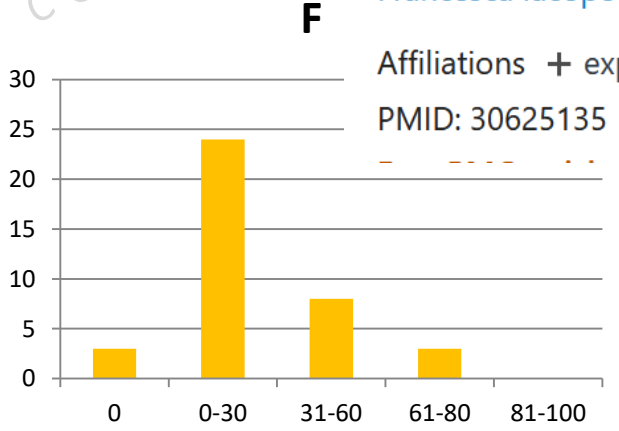
A cross-sectional serosurvey in a sheep population in central Italy following a bluetongue epidemic

Andrea Carvelli [1], Marcello Sala [1], Gian Luca Autorino [2], Maria Teresa Scicluna [2], Francesca Iacoponi [1], Pasquale Rombolà [1], Paola Scaramozzino [1]

Affiliations + expand

PMID: 30625135 PMCID: PMC6326410 DOI: 10.1371/journal.pone.0208074





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CONSIDERAZIONI CONCLUSIVE

● LAZIO e TOSCANA in restrizione per BTV1 e BTV4

biennio 2019-2020 circolazione virale limitata

Nel Lazio – fine 2020 – molti focolai clinici

● nuova normativa prevede condizioni per movimentare gli ovini molto meno restrittive

eliminate le restrizioni bovini e caprini

favoriti scambi commerciali, ma potenziale diffusione

● sorveglianza sierologica ed entomologica gestita con alcune difficoltà

● vaccinazione volontaria e a carico dell'allevatore (costi/benefici)

polizze assicurative?

● L'ampia variabilità delle caratteristiche delle epidemie dal 2013 al 2020 e l'assenza di associazione con campagne vaccinali o biosicurezza aziendale, rendono difficile l'individuazione di trend utili all'applicazione di strategie di controllo

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AHS e BT

- Article 6
- When BT presence is confirmed, the official veterinarian shall proceed with the slaughter deemed necessary to prevent extension of the epidemic

Whereas:

(1) In accordance with Article 15 of Directive 92/119/EEC, specific measures to control and eradicate bluetongue should be introduced.

(2) The epidemiological characteristics of bluetongue are comparable to those of African horse sickness.

(3) The Council has adopted Directive 92/35/EEC laying down control rules and measures to combat African horse sickness (2).

(4) To combat bluetongue, therefore, the overall measures laid down by Directive 92/35/EEC to combat African horse sickness should be used as a model, making appropriate adjustments due to the characteristics of the rearing of species susceptible to bluetongue.

(5) Rules should be laid down on the movement of susceptible species and their semen, ova and embryos from areas subject to restrictions arising from an outbreak of the disease.

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CONSIDERAZIONI CONCLUSIVE

Nuovo approccio è un depotenziamento

Necessity of 1 policy in EU but very different MS situations. 3 zones: epidemic/mid/endemic

Non più esotica in Med basin, non si parli di eradicazione

Chiarire il ruolo dei determinant antropogenici di malattia BTV 8, 6, 11

Informare allevatori del cambio di strategia

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CONSIDERAZIONI CONCLUSIVE

- Bt is a problem with many dimensions:
  - Production
  - Veterinary
  - Scientific
  - Environmental
  - Climatic
  - Economic
  - Trade
  - Political

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Thanks for your attention

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