



CESME

Centro di Referenza Nazionale

Giornata di studio sulla BlueTongue



L'enigma della variabilità clinica nella
Bluetongue: da asintomatica a letale

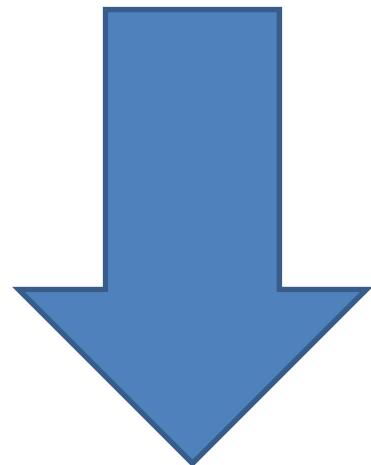


Istituto Zooprofilattico
Sperimentale della Sardegna

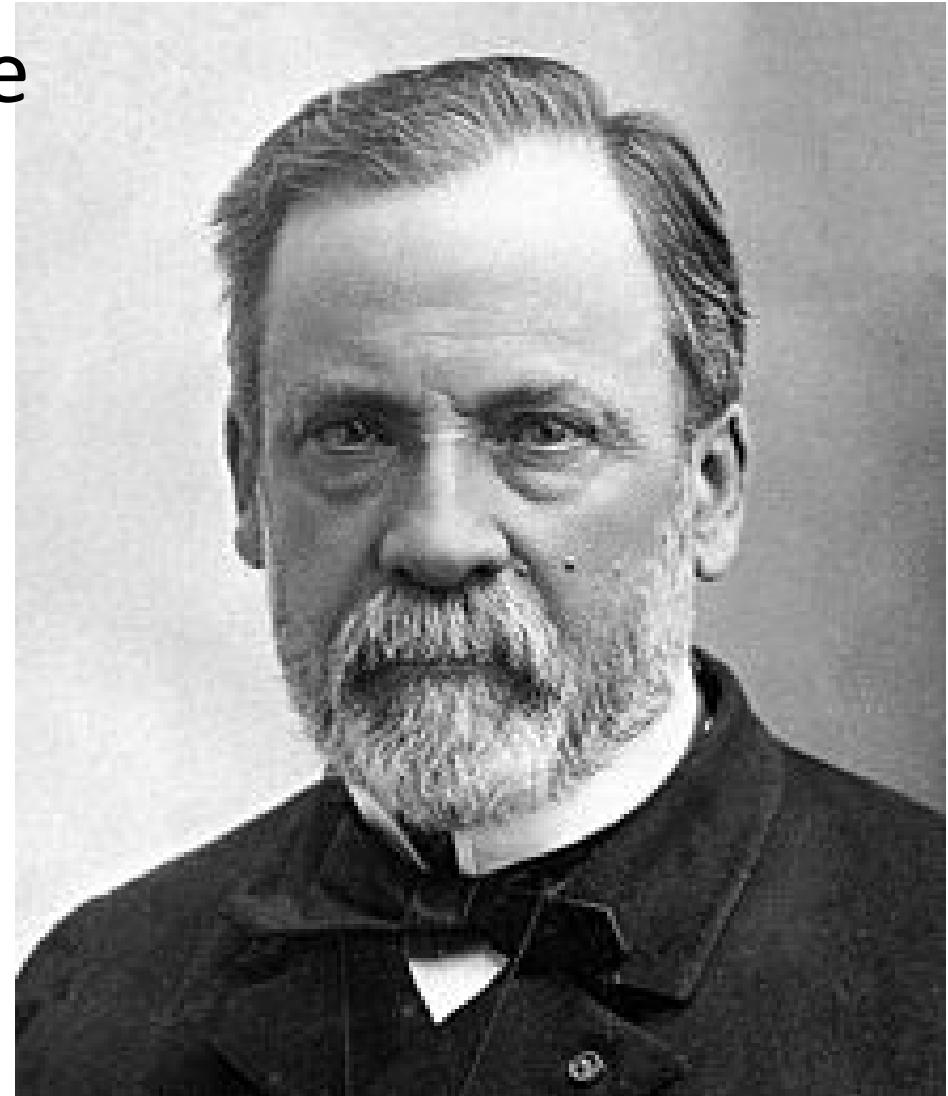
Ciriaco Ligios, Giantonella Puggioni

The Pasteur's era and the
Koch's postulates

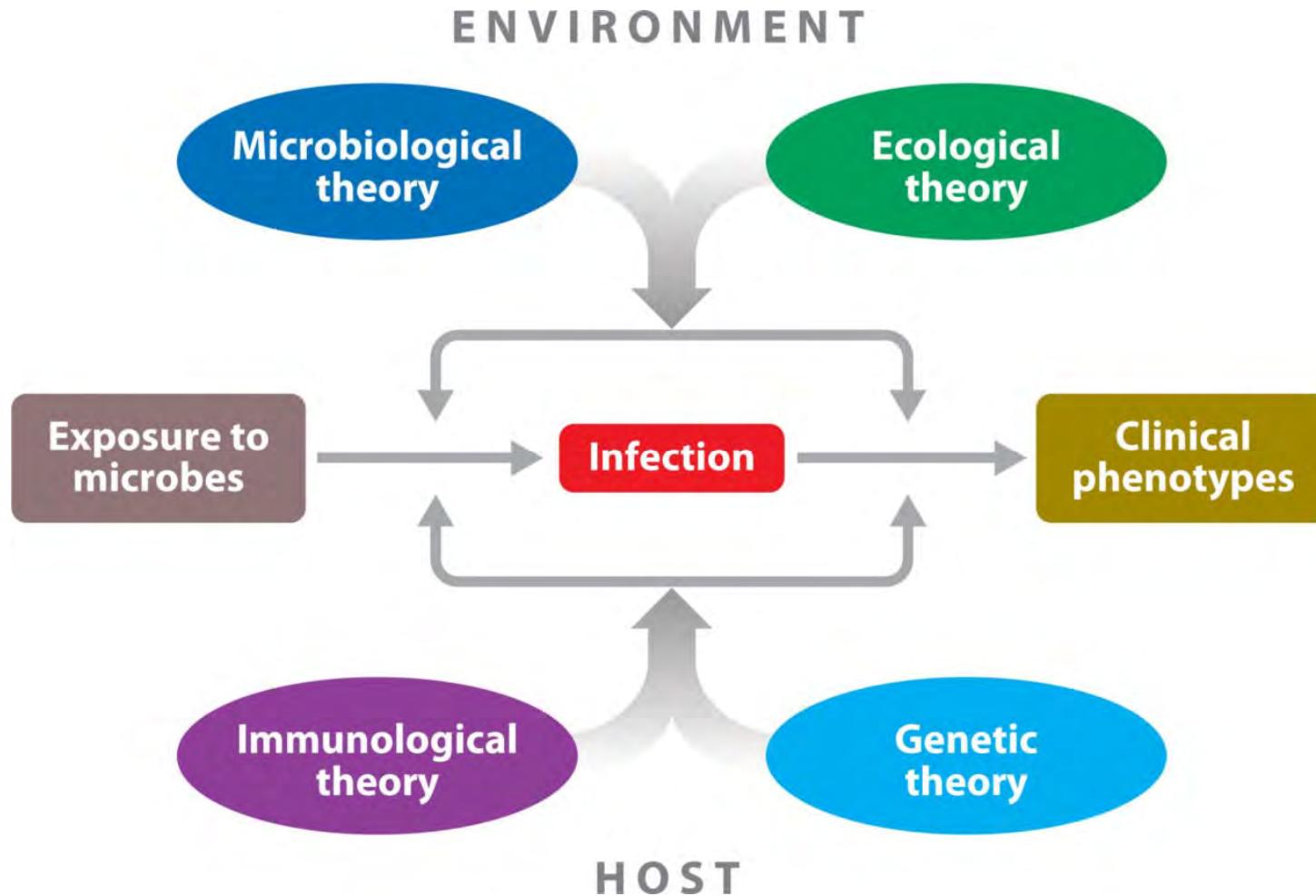
One germ



One disease

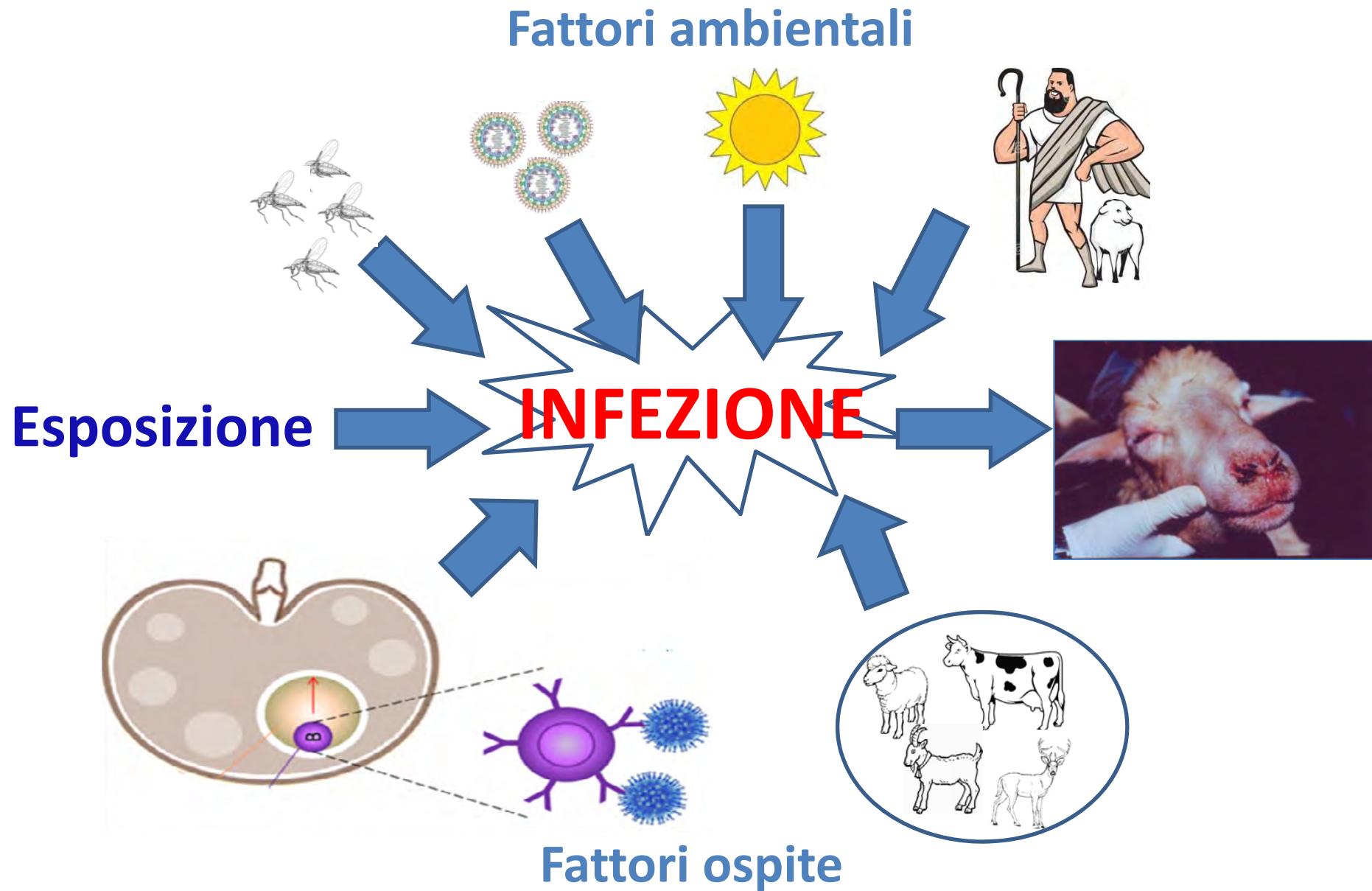


How explain the enigma of the clinical variability between individuals in the course of infectious disease ?



Casanova et al. Annu Rev Genomics Hum Genet. 2013 ; 14: 215–243

Perché tanta variabilità nella manifestazione clinica della BlueTongue ?

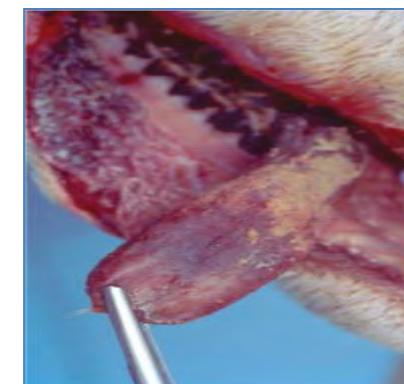


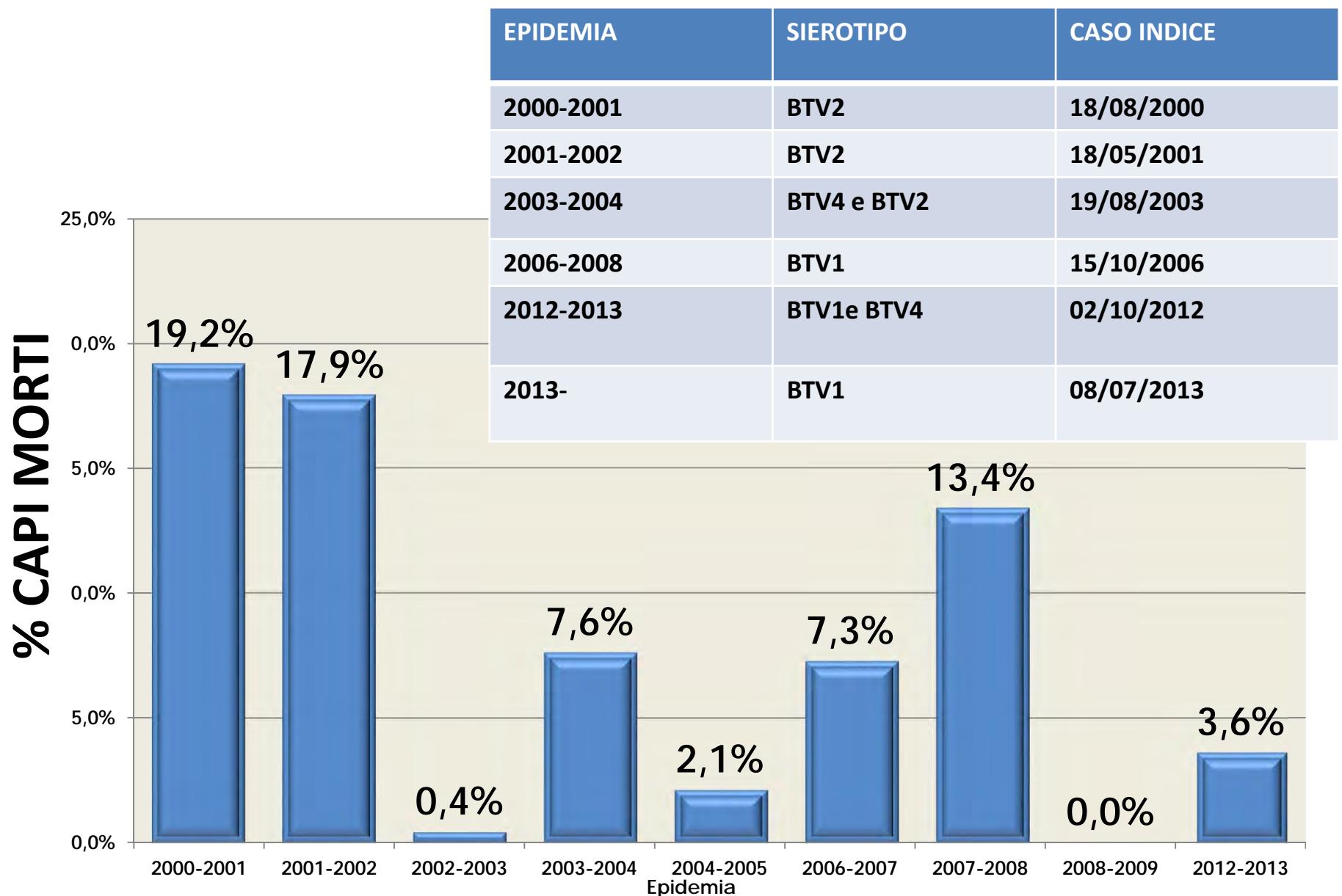
L a patogenesi della BT

**There are marked differences in the severity of
the disease that occurs in different species and
breeds after BTV infection, and infection of the
same specie or breeds sheep with different
virus strains.**

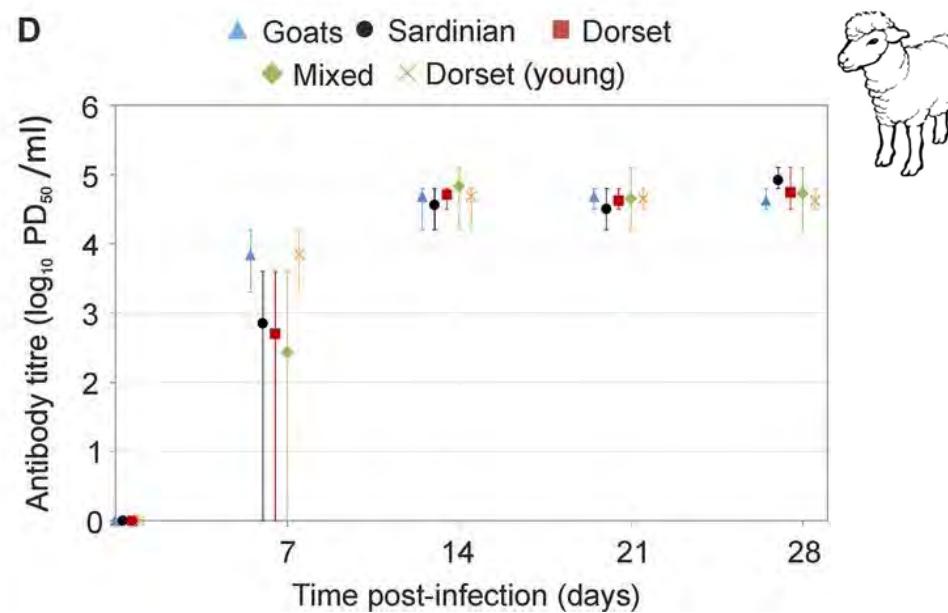
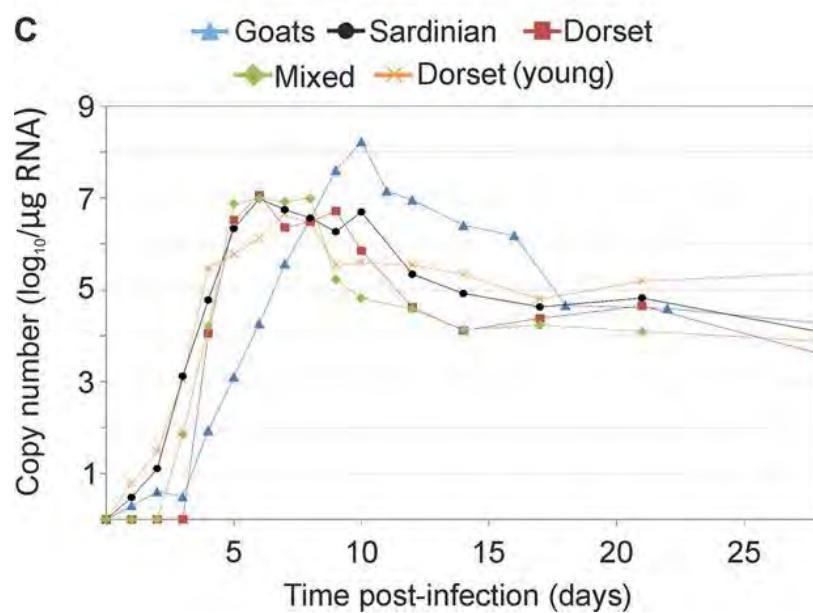
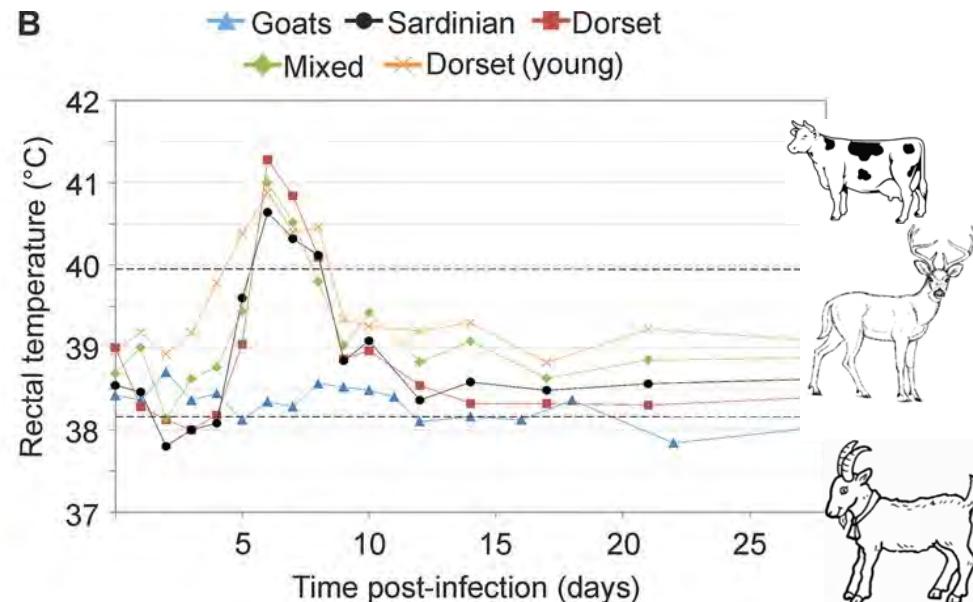
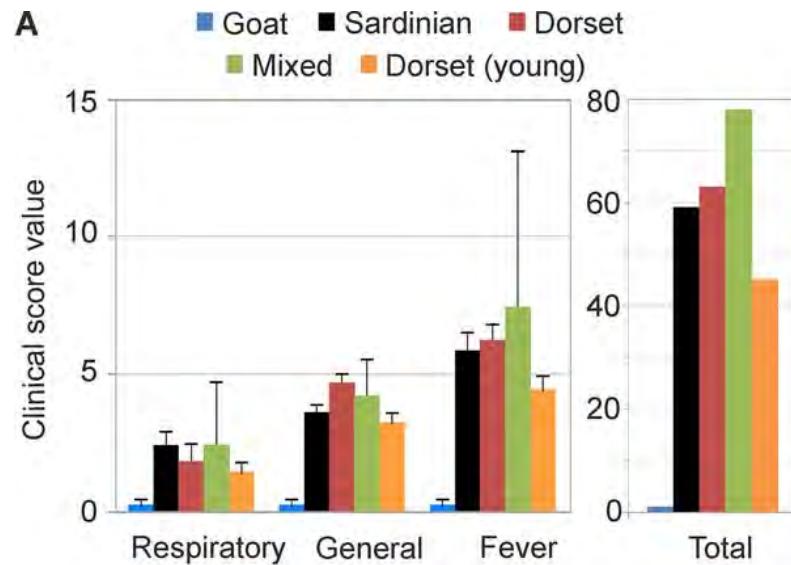
MacLachan J.Comp.Path. 2010

Quadri clinici di BTV

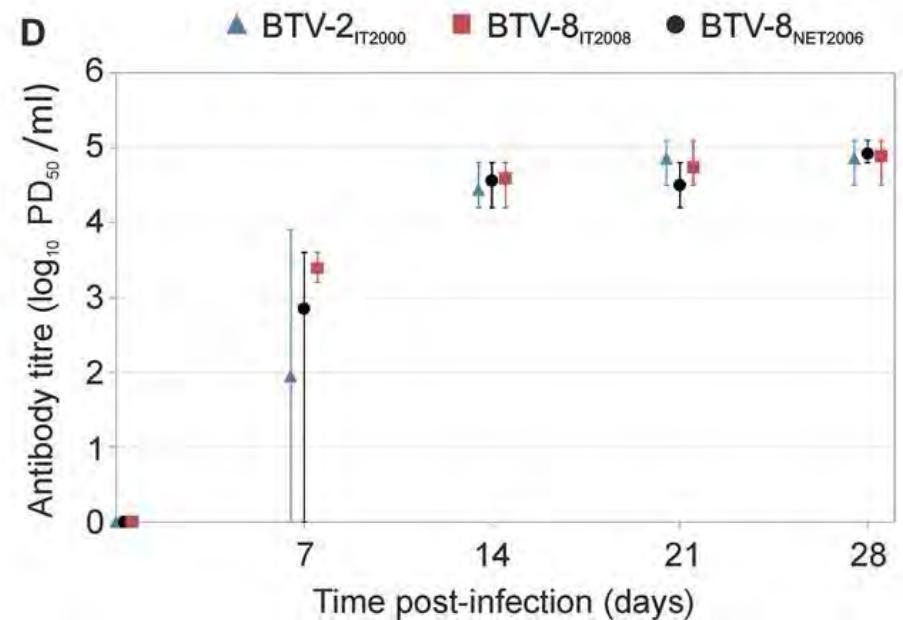
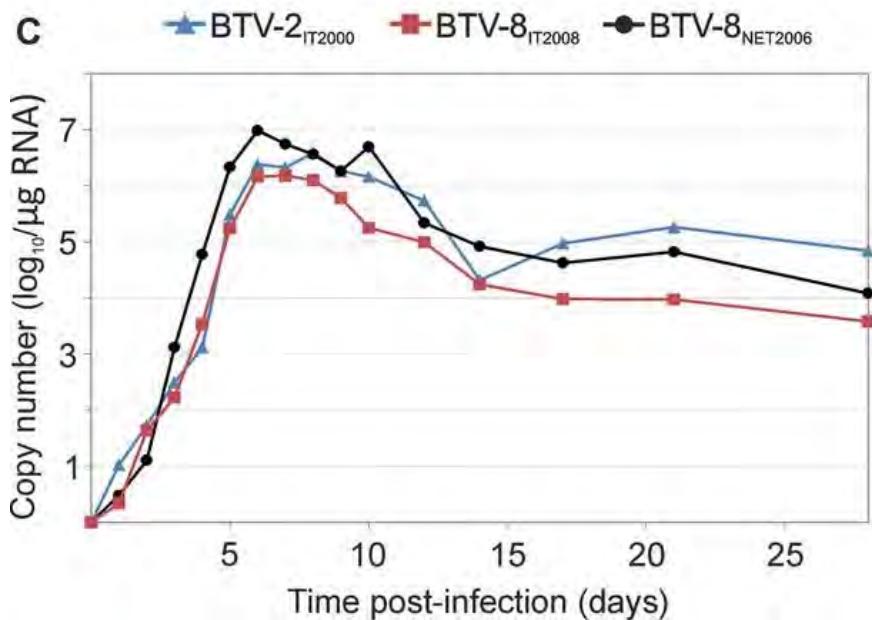
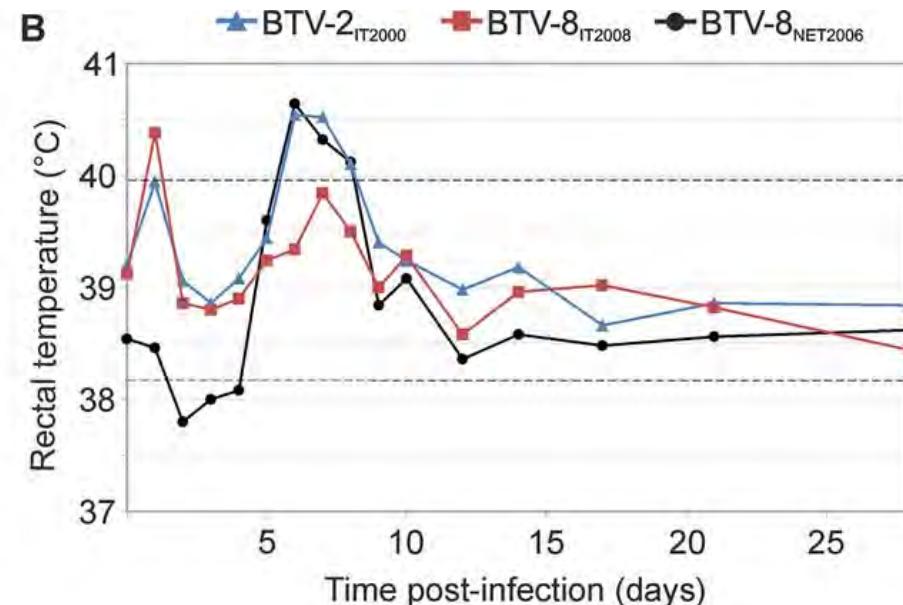
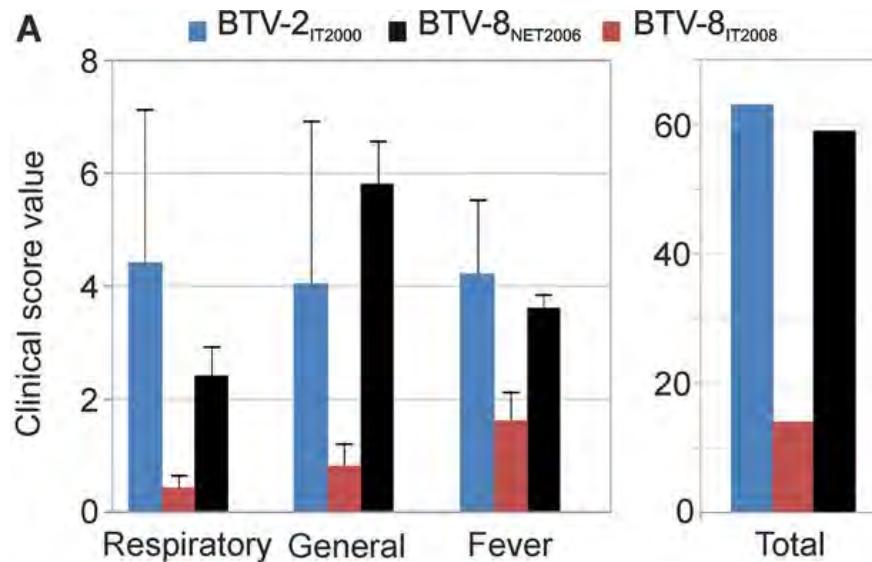




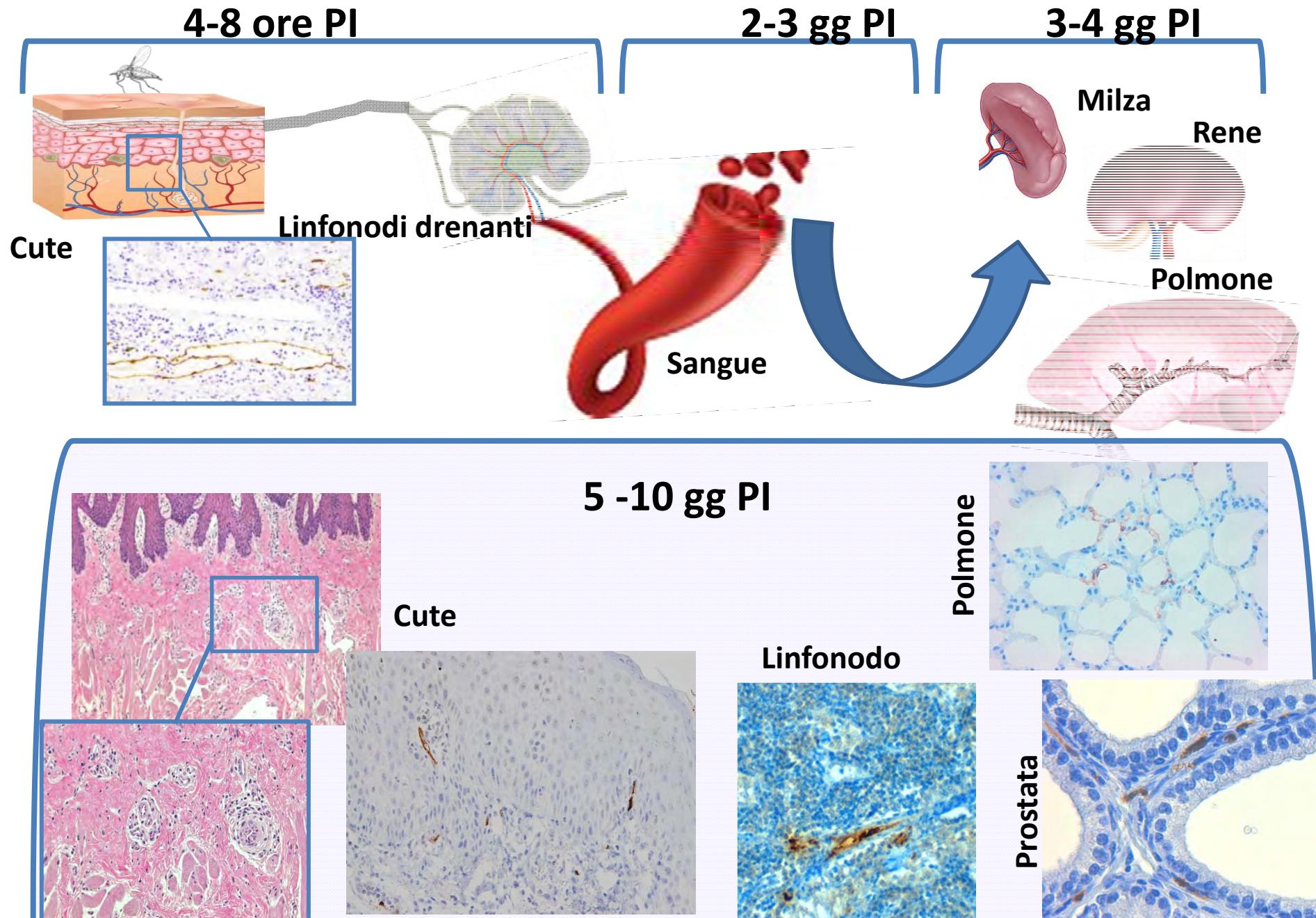
Experimental infection of goats and different sheep breeds with BTV-8NET2006.



Virulence of BTV-2_{IT2000}, BTV-8_{NET2006}, and BTV-8_{IT2008}.



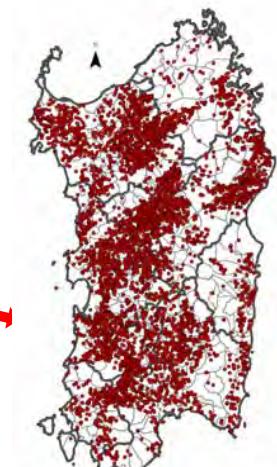
PATOGENESI DELLA BLUETONGUE



Tra i fattori legati al virus, come sierotipo ed isolato, quale è il più importante nel determinare il fenotipo clinico della malattia ?

Per questo abbiamo utilizzato 2 isolati BTV1 :

BTV1₂₀₀₆ and **BTV1₂₀₁₃** rispettivamente
dell'epidemia del 2006 e del 2013 in Sardegna.



Animali utilizzati

N. 6 montoni con BTV1₂₀₀₆ e N. 6 con BTV1₂₀₁₃



BTV1₂₀₀₆



BTV1₂₀₁₃

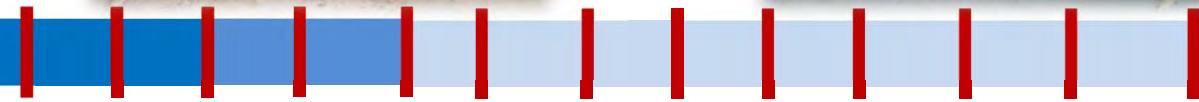


Il sangue è stato inoculato s.c. e i.d.

Autorizzazione: N. 1248/2015-PR

Esperimento

BTV1₂₀₁₃



5 gg p.i.



15 gg p.i

BTV1₂₀₁₃



Follow-up clinico, sierologico e virologico

CONTROLLO A 5 GG *p.i.*

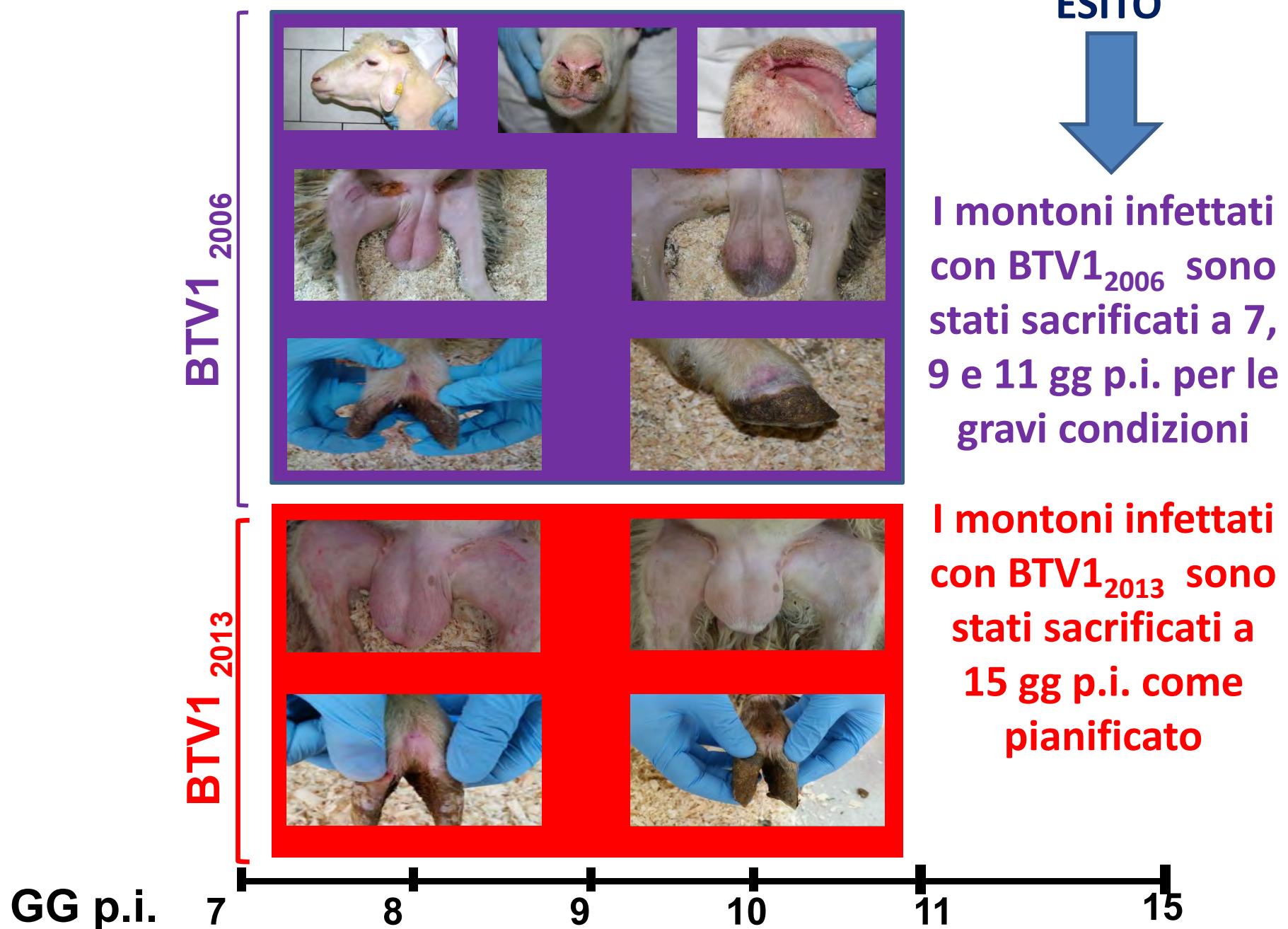
BTV1₂₀₁₃



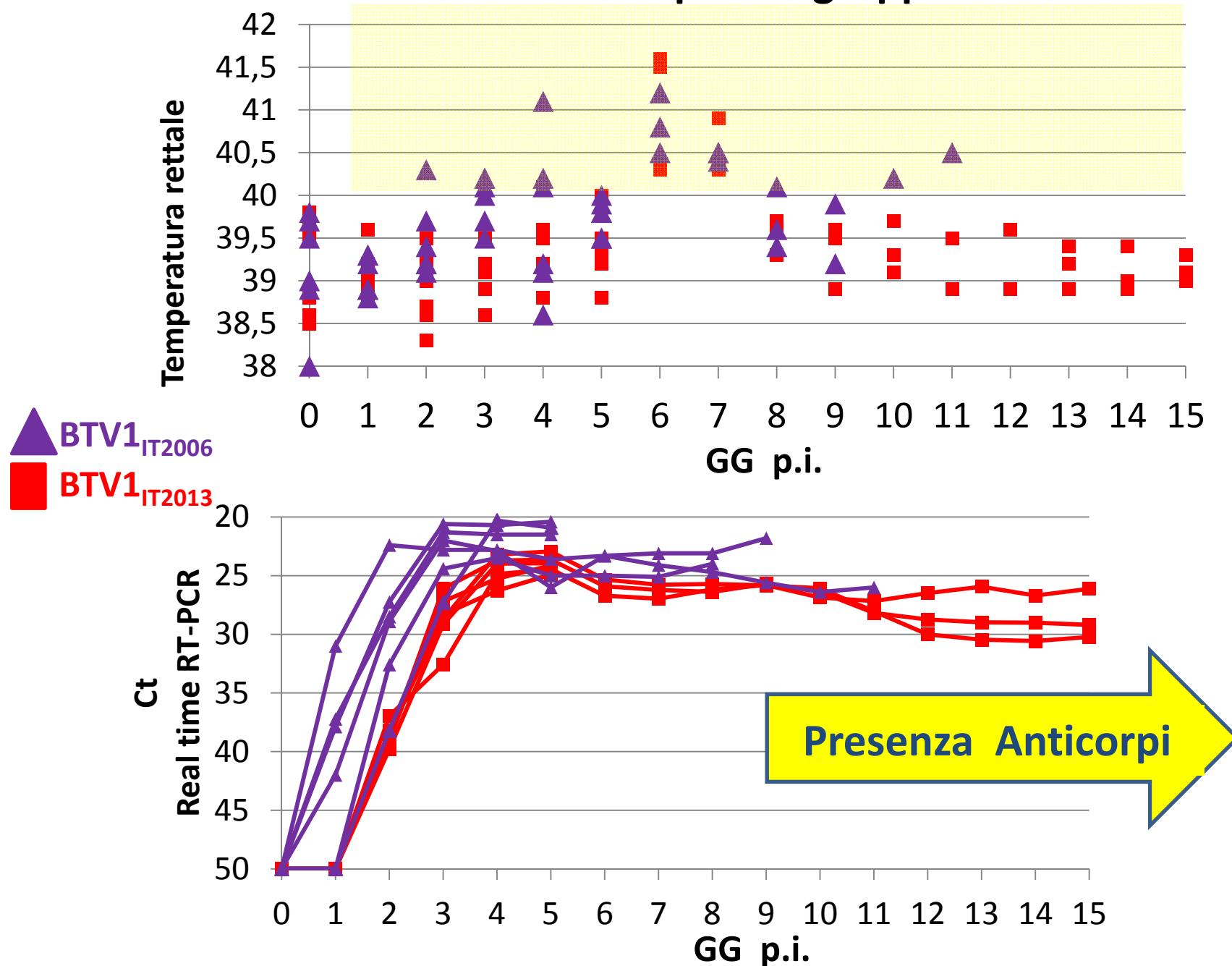
BTV1₂₀₁₃

Nessun segno clinico nei montoni esaminati a 5 gg p.i.

RISULTATI TRA 5 E 15 GG p.i.



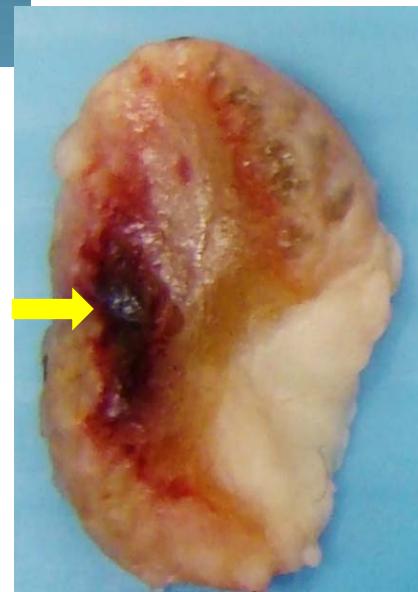
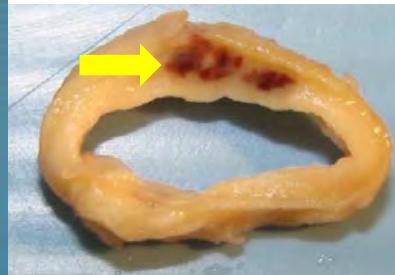
Follow-up nei 2 gruppi



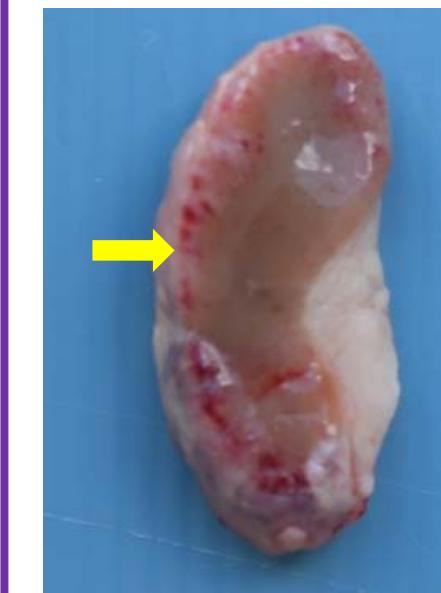
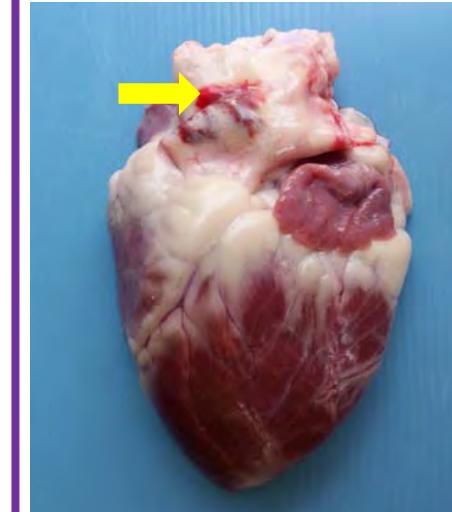
BTV1₂₀₁₃

LESIONI ANATOMOPATOLOGICHE

BTV1₂₀₀₆



Lesioni in montoni sacrificati
a 15 gg p.i.



Lesioni in montoni a 7,9 e 11
gg p.i.

BTV1 / 2006

PUNTEGGIO CLINICO	GIORNI POST-INOCULUM										
	1	2	3	4	5	6	7	8	9	10	11
Edema faciale											
Congestione/edema cute											
Congestione/edema dello scroto											
Appetito											
Difficoltà locomotorie											
Scolo nasale/dispnea											
Salivazione											
Congestione emorragie bocca/musello											

BTV1 / 2013

	12	13	14	15
Edema faciale				
Congestione/edema cute				
Congestione/edema dello scroto				
Appetito				
Difficoltà locomotorie				
Scolo nasale/dispnea				
Salivazione				
Congestione emorragie bocca/musello				

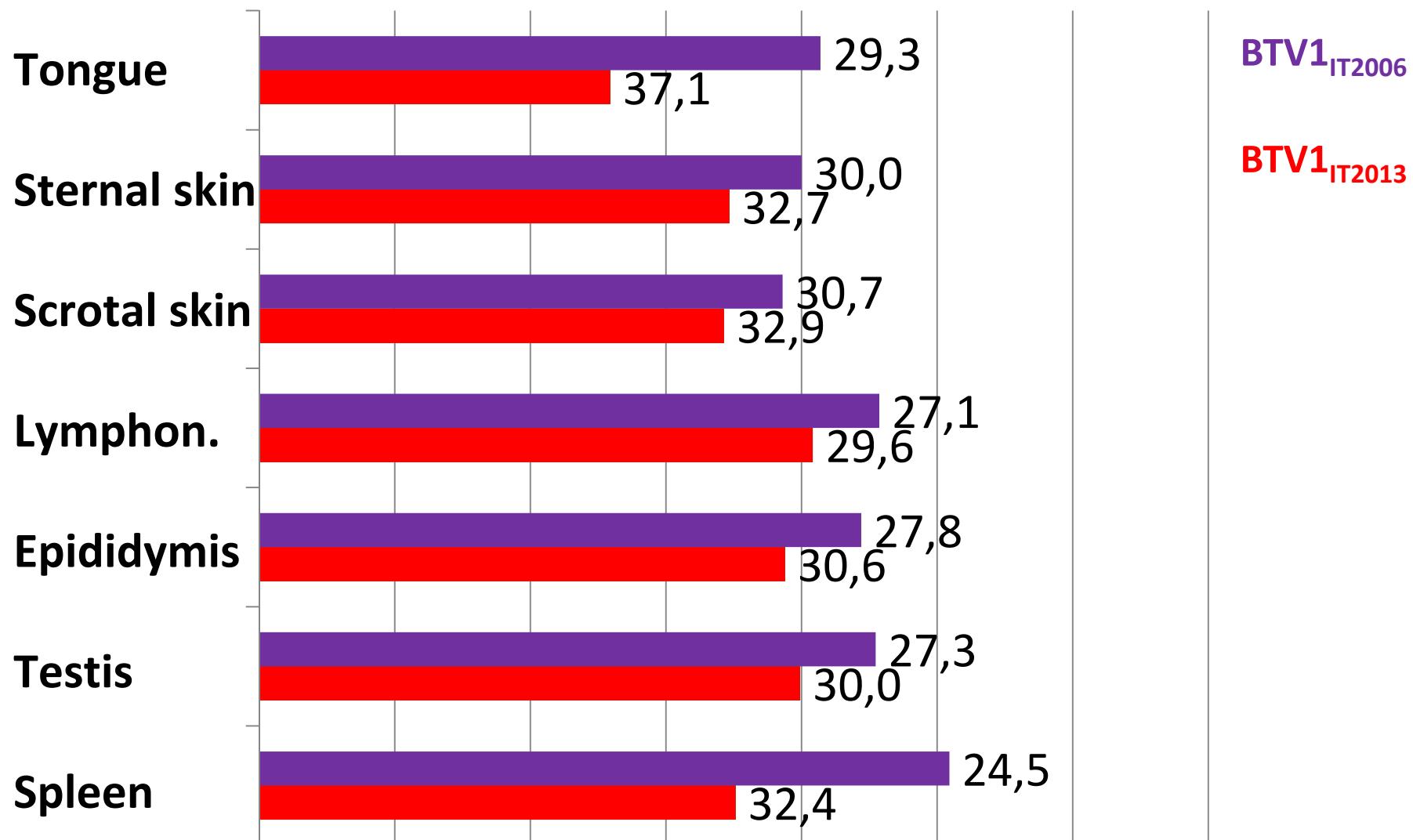
Normale

Lieve

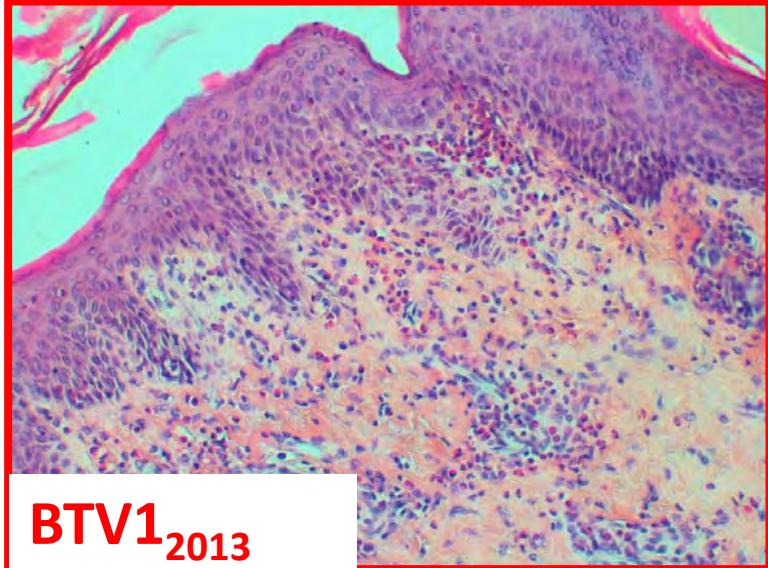
Moderata

Grave

Valori di RNA virale con Real time RT-PCR (ct)

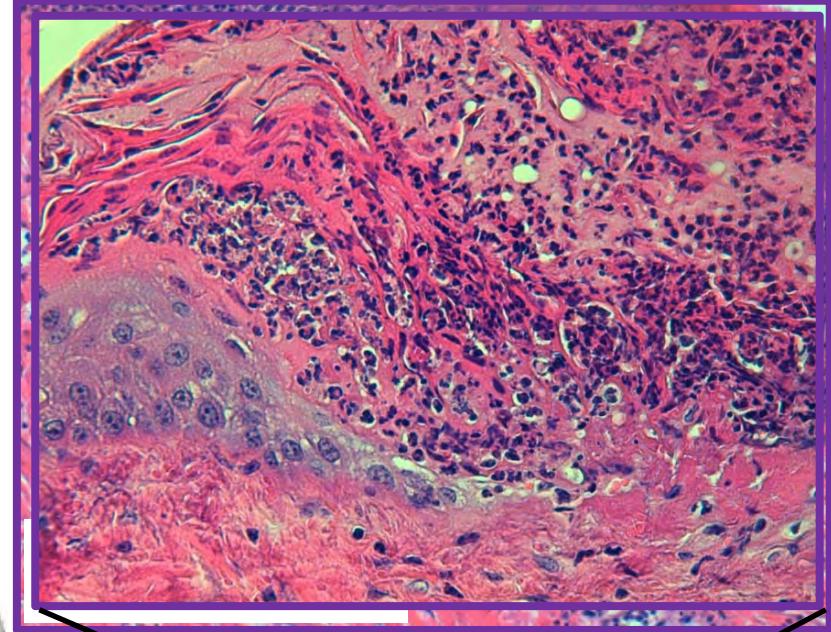


Cute: lesioni istologiche con BTV1₂₀₁₃ BTV1₂₀₀₆

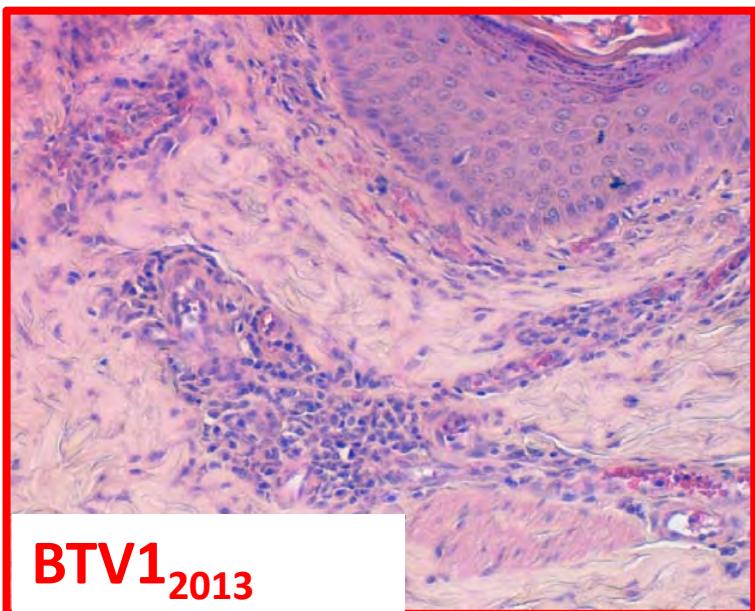


BTV1₂₀₁₃

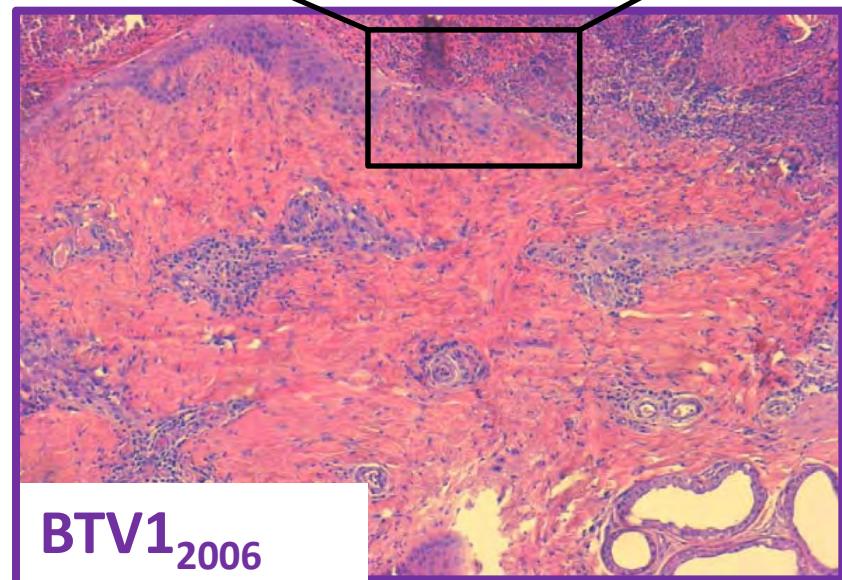
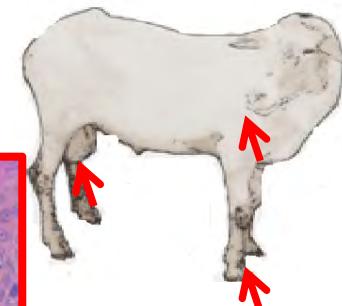
5 gg p.i.



7-9-11 gg p.i.

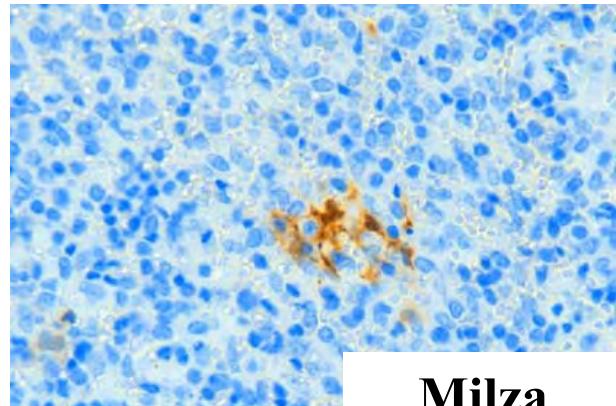


15 gg p.i.

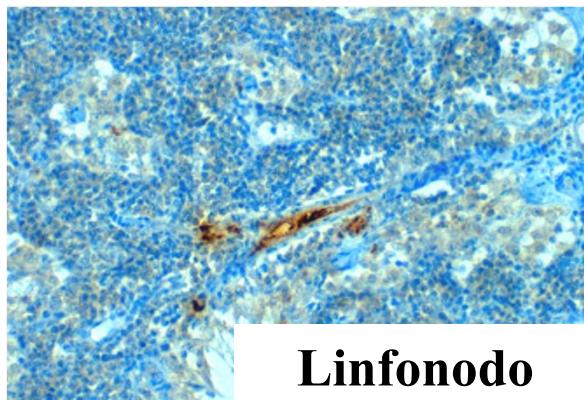


BTV1₂₀₀₆

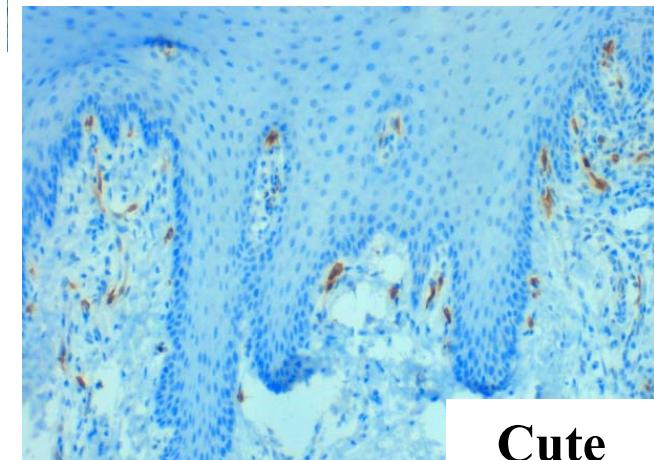
Evidenziazione della proteina NS2 nel corso di BT a 5-7 gg p.i.



Milza

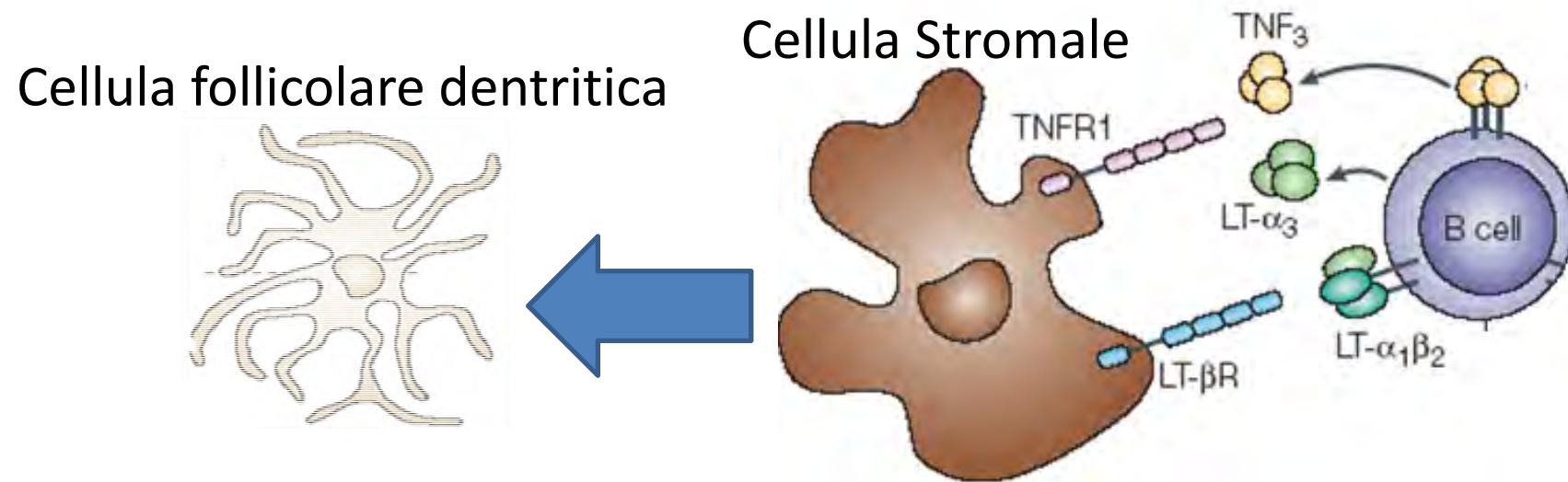


Linfonodo



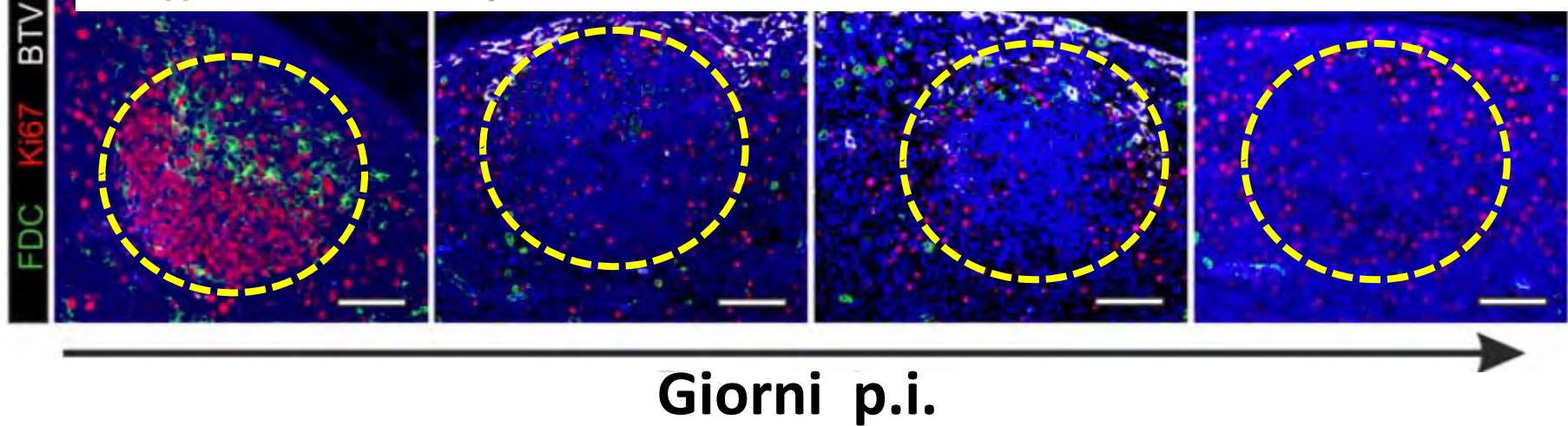
Cute

BTV e cellule follicolari dendritiche



Follicular dendritic cell disruption as a novel mechanism of virus-induced immunosuppression

Eleonora Melzi^a, Marco Caporale^{a,b}, Mara Rocchi^c, Verónica Martín^d, Virginia Gamino^e, Andrea di Provvido^b, Giuseppe Marruchella^{b,f}, Gary Entrican^c, Noemí Sevilla^d, and Massimo Palmarini^{a,1}

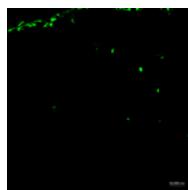


Confocal immunofluorescence: different outcomes on the two BTV1 isolates in the lymphonode

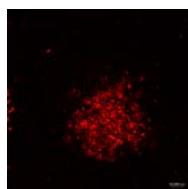
BTV1₂₀₁₃ infection at 5 days p.i



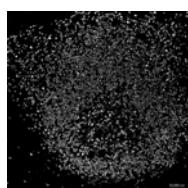
DAPI



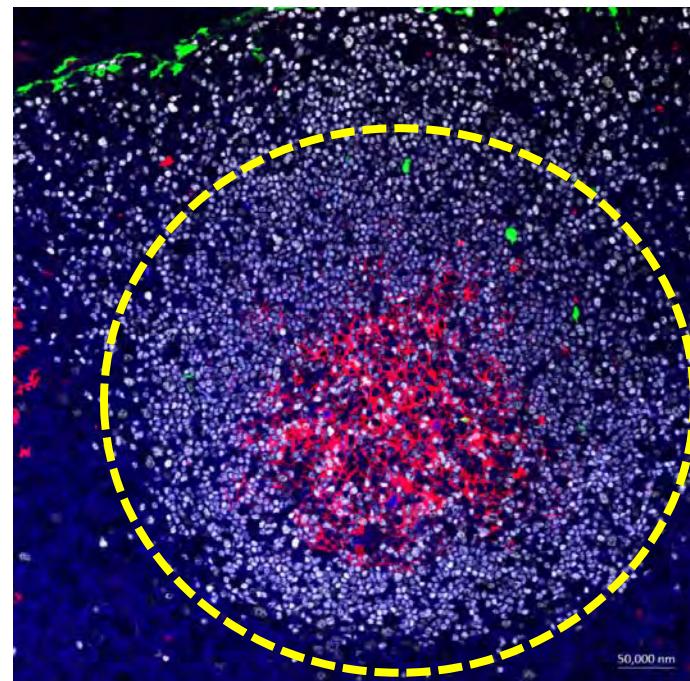
NS2 BTV



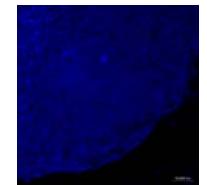
CNA42



PAX5



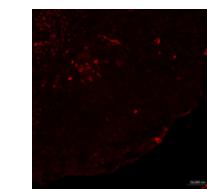
BTV1₂₀₀₆ infection at 5 days p.i



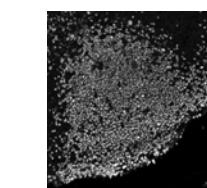
DAPI



NS2 BTV



CNA42

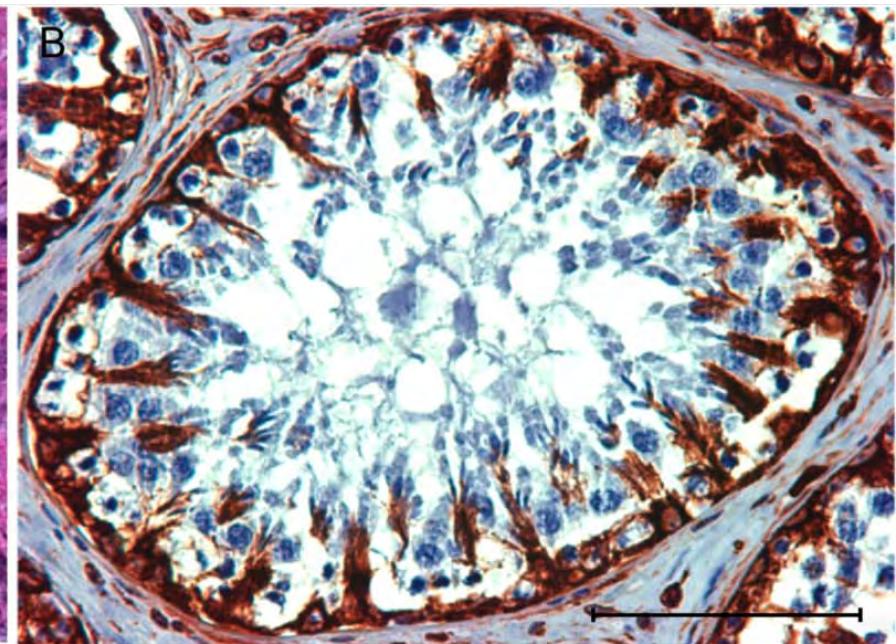
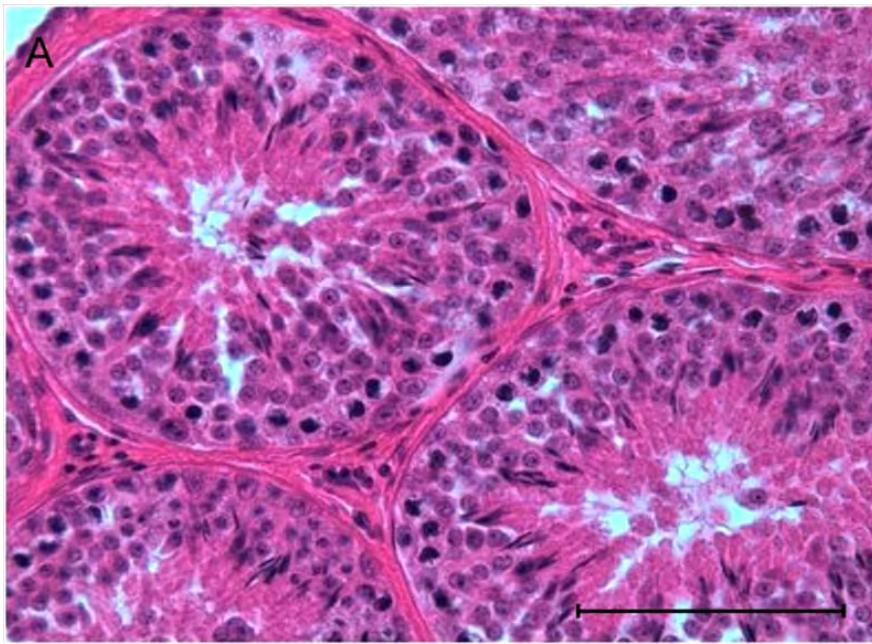


PAX5

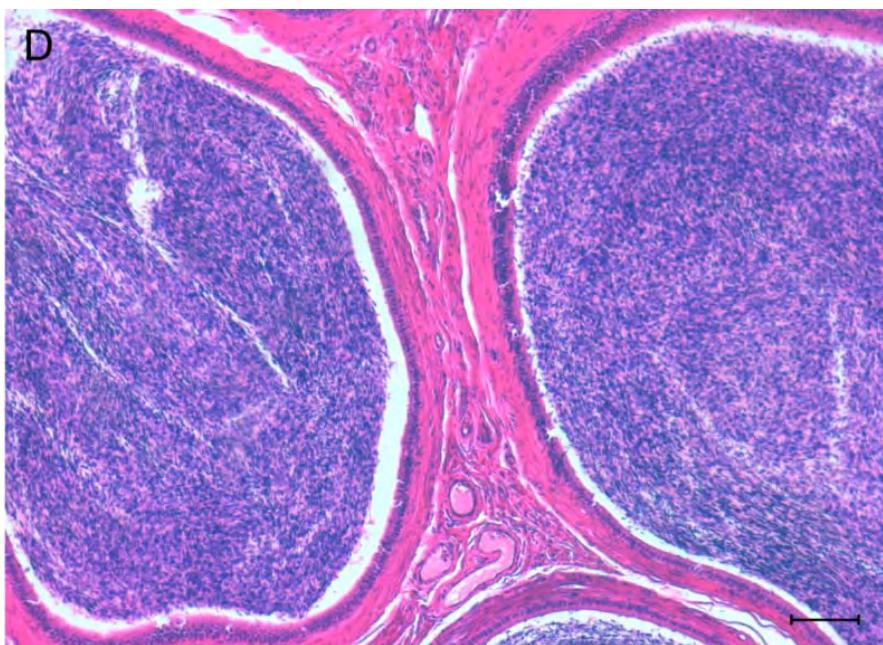
DAPI: Nuclear Counterstain
NS2: BT virus
CNA42: Follicular dendritic cells
PAX5: Lymphocytes B

ISTOLOGIA DEL TESTICOLO E DELL'EPIDIDIMO SANO

TESTICOLO

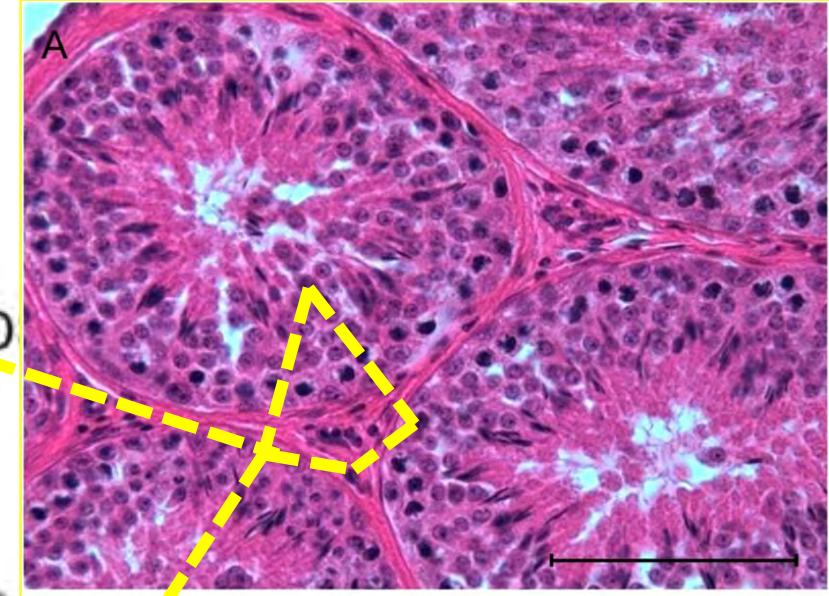
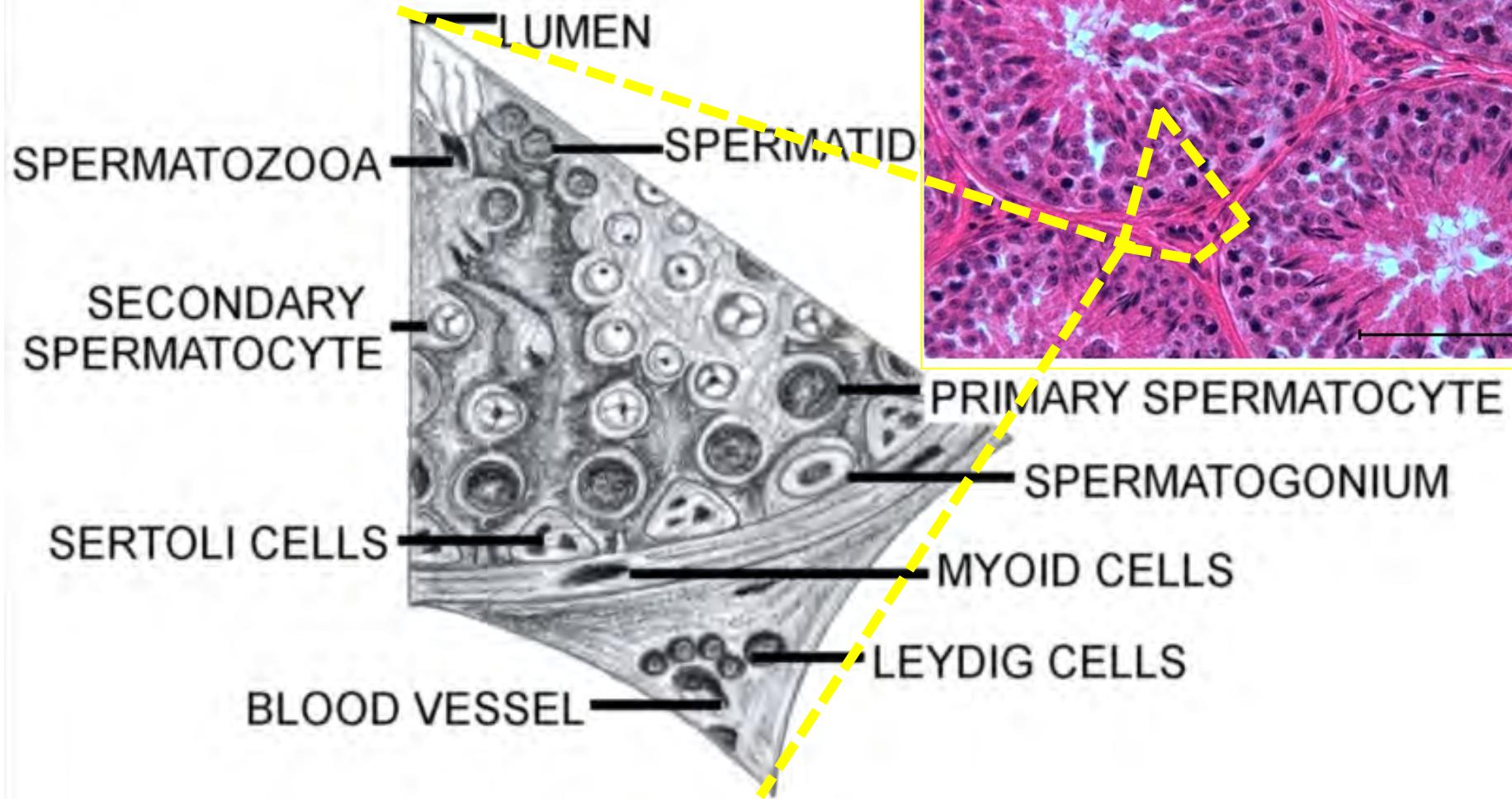


EPIDIDIMO



Testicolo normale

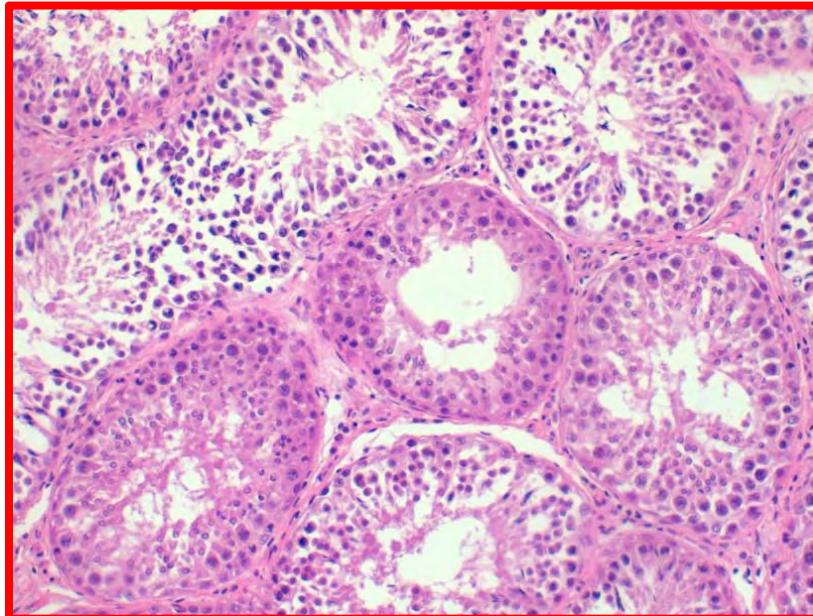
C



TESTIS: histological lesions in BTV1₂₀₁₃ and BTV1₂₀₀₆ rams

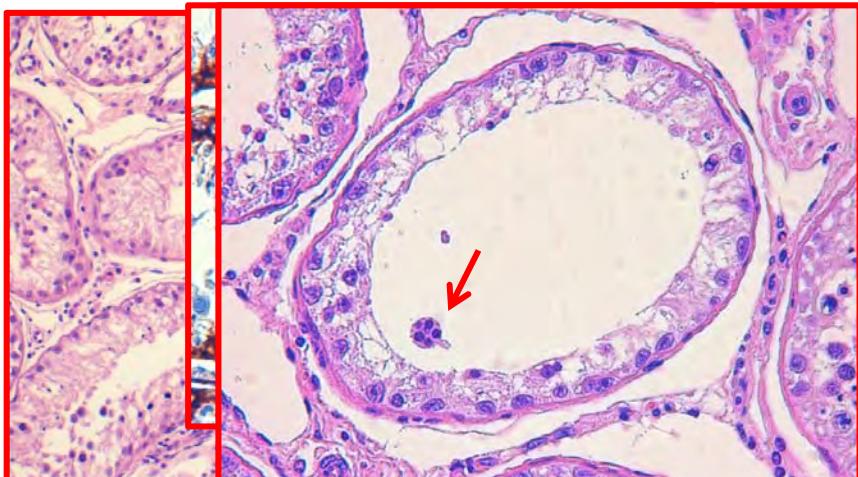
5 days p.i..

BTV1₂₀₁₃



15 days p.i.

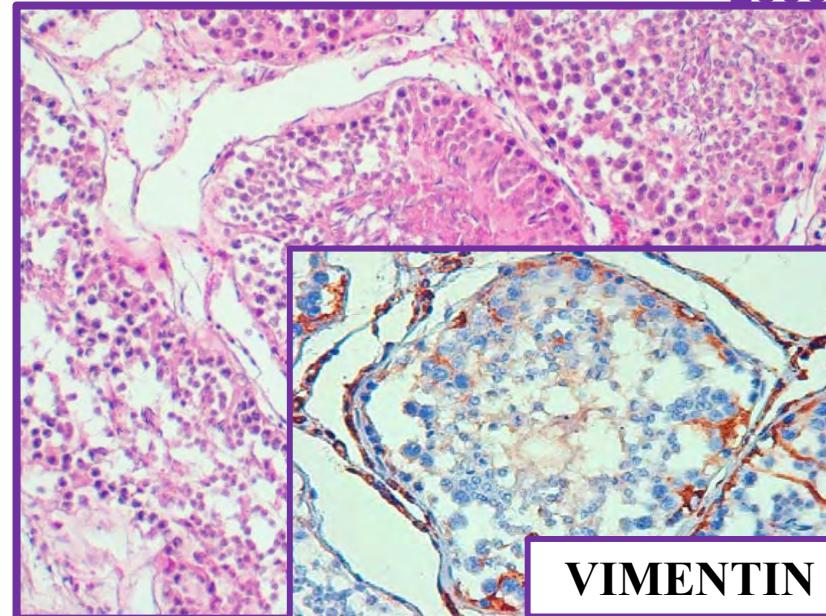
BTV1₂₀₁₃



VIMENTIN

5 days p.i..

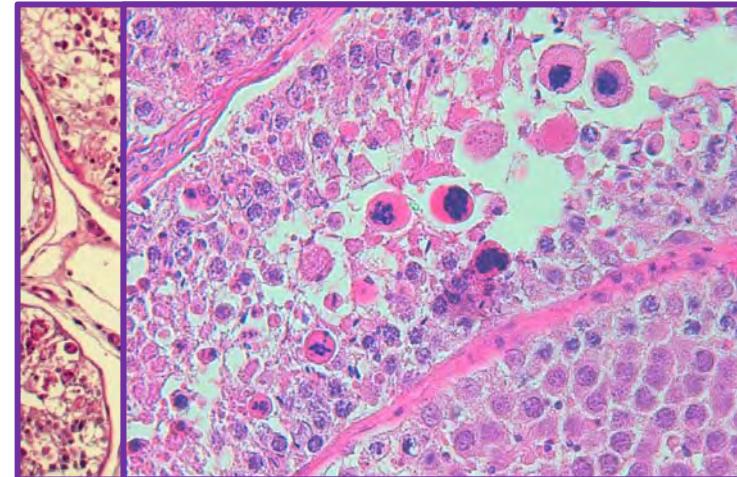
BTV1₂₀₀₆



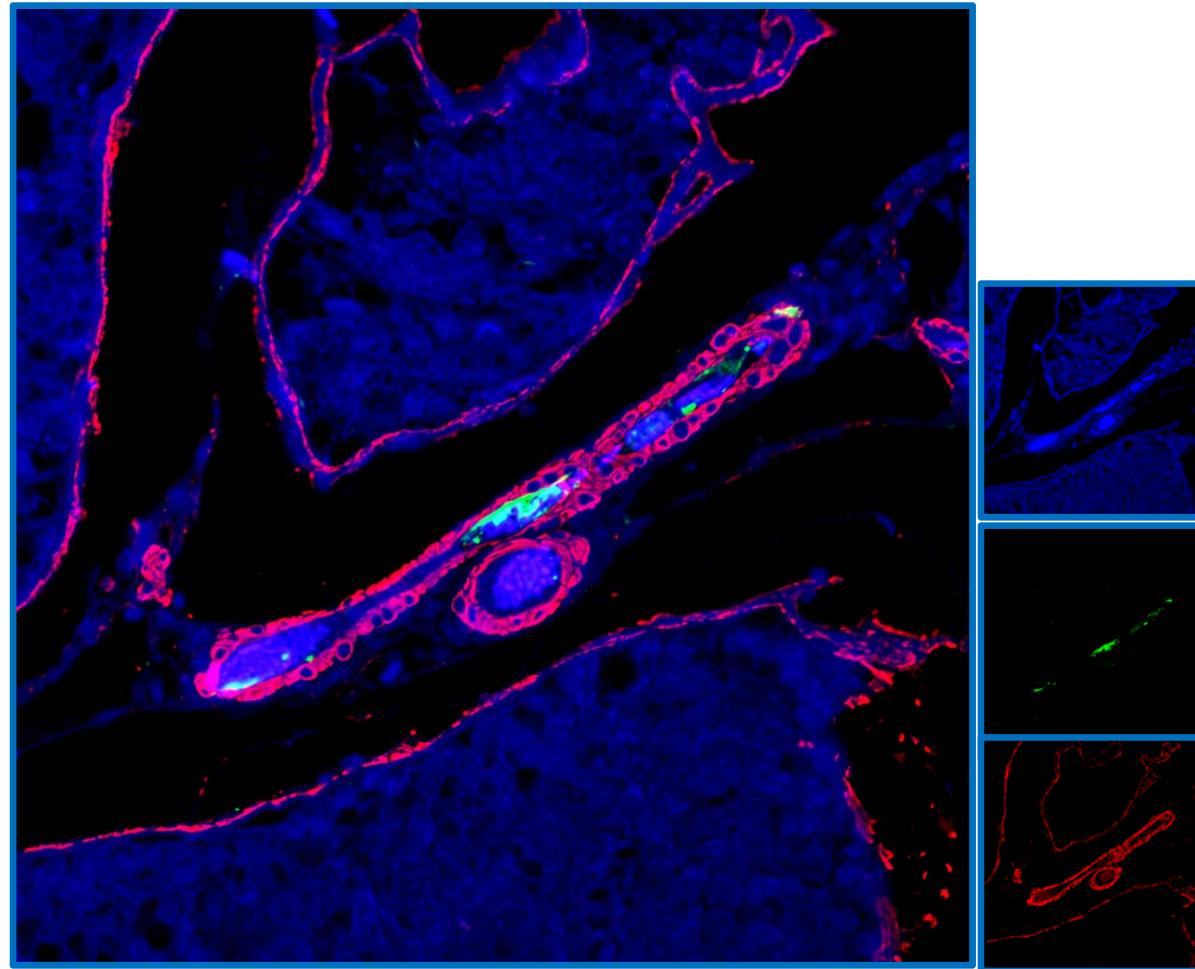
VIMENTIN

7-9-11 days p.i.

BTV1₂₀₀₆



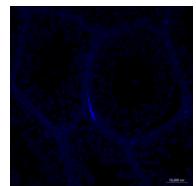
Localizzazione di BTV mediante immunofluorescenza



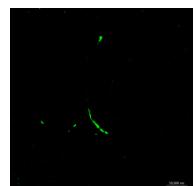
DAPI: Nuclear Counterstain; NS2: BT V; SMA: Smooth muscle actin

Confocal immunofluorescence: different outcomes of the two BTV1 isolates on the testis

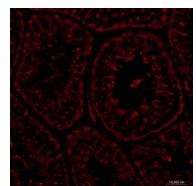
BTV1₂₀₁₃ infection at 5 days p.i



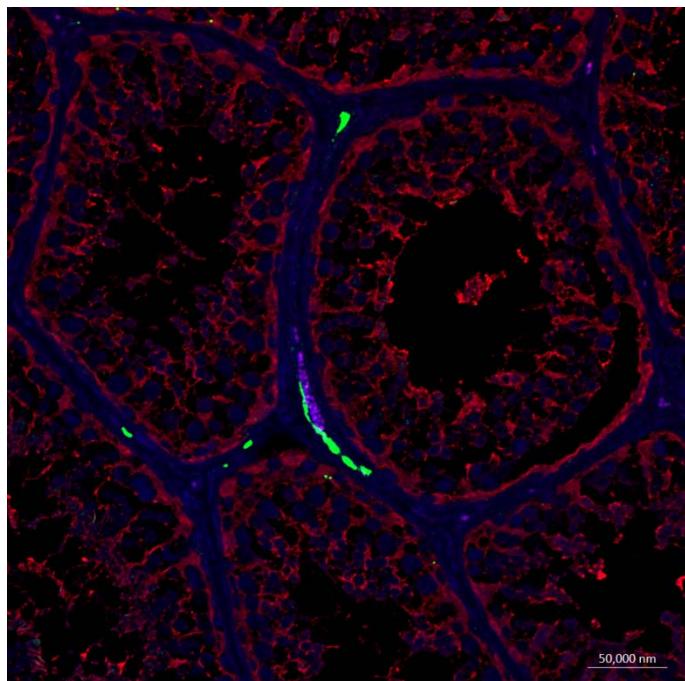
DAPI



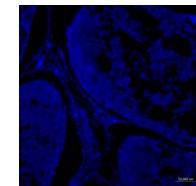
NS2 BTV



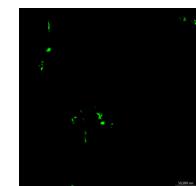
S 100



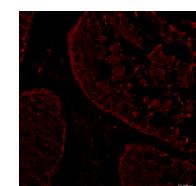
BTV1₂₀₀₆ infection at 5 days p.i



DAPI



NS2 BTV

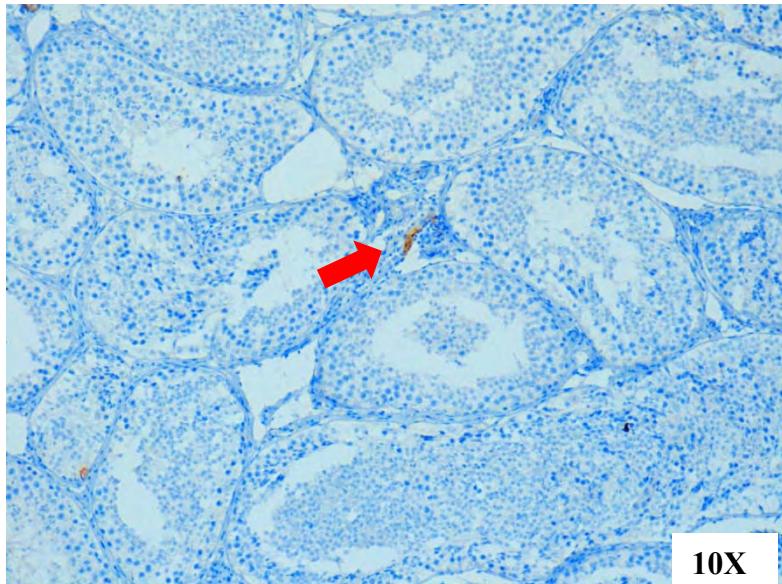


S 100

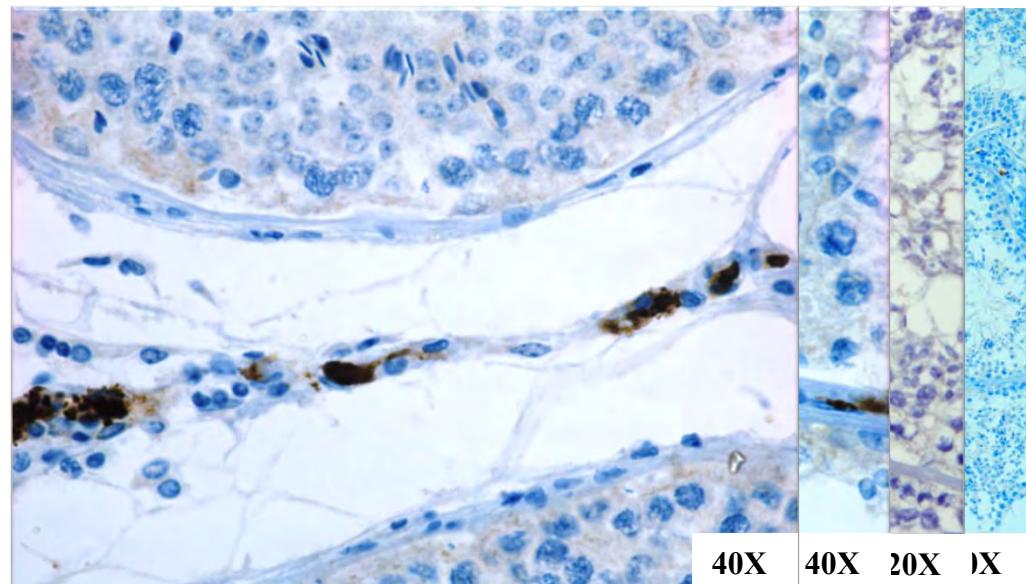
DAPI: Nuclear Counterstain
NS2: BT virus
S 100: Sertoli's cells

Localizzazione di BTV – 1 /2013 mediante immunoistochimica (NS2)

5 GG P.I.



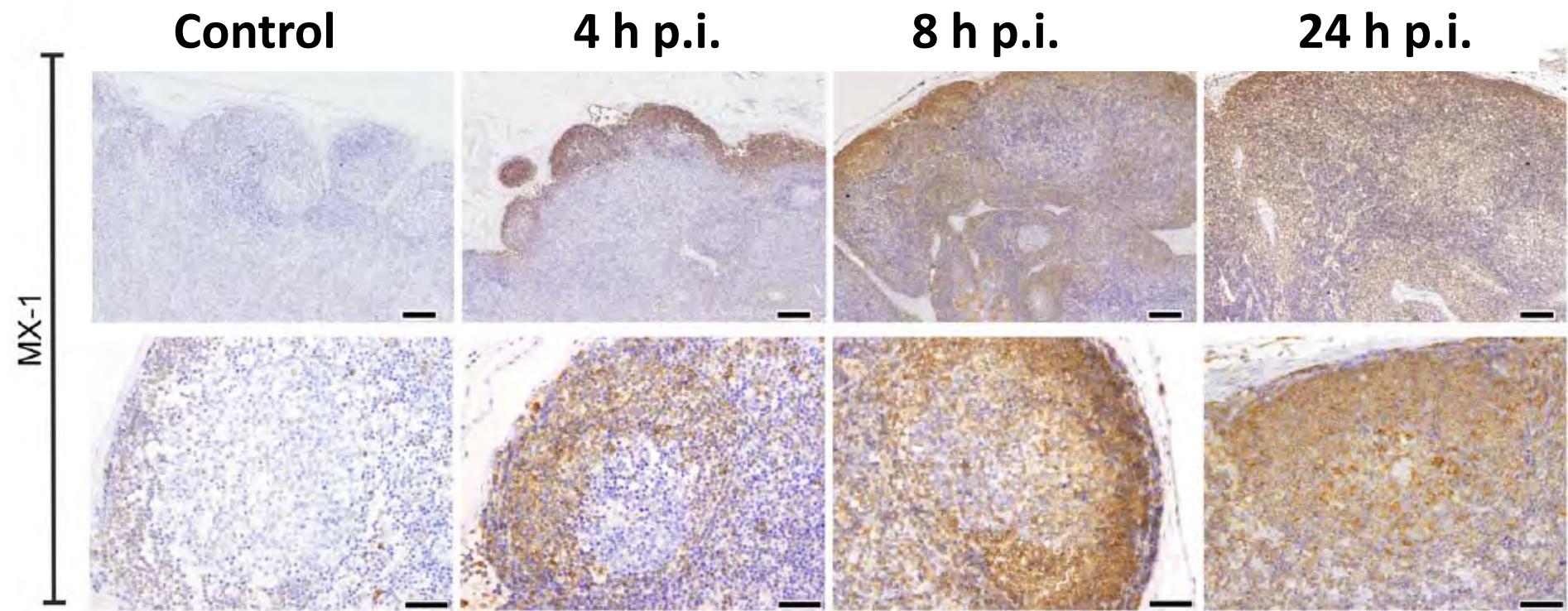
7 GG P.I.



15 GG P.I.



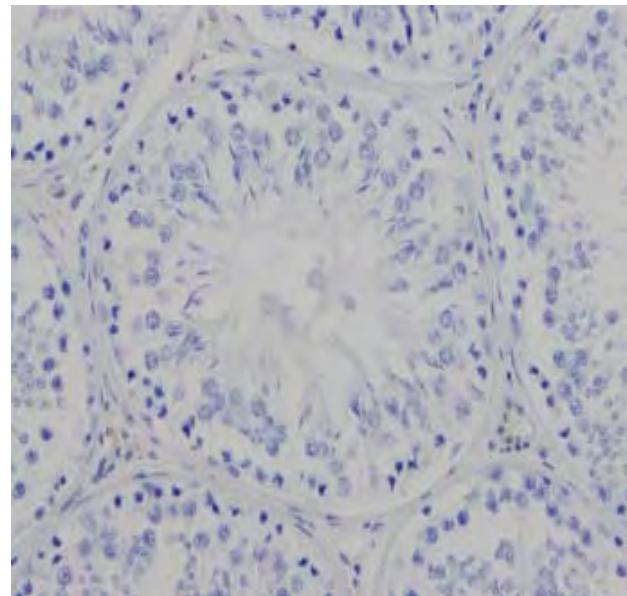
Produzione di proteina Mxi nel linfonodi periferici satelliti dopo somministrazione sperimentale di BTV per via intradermica (Melzi et al. 2016)



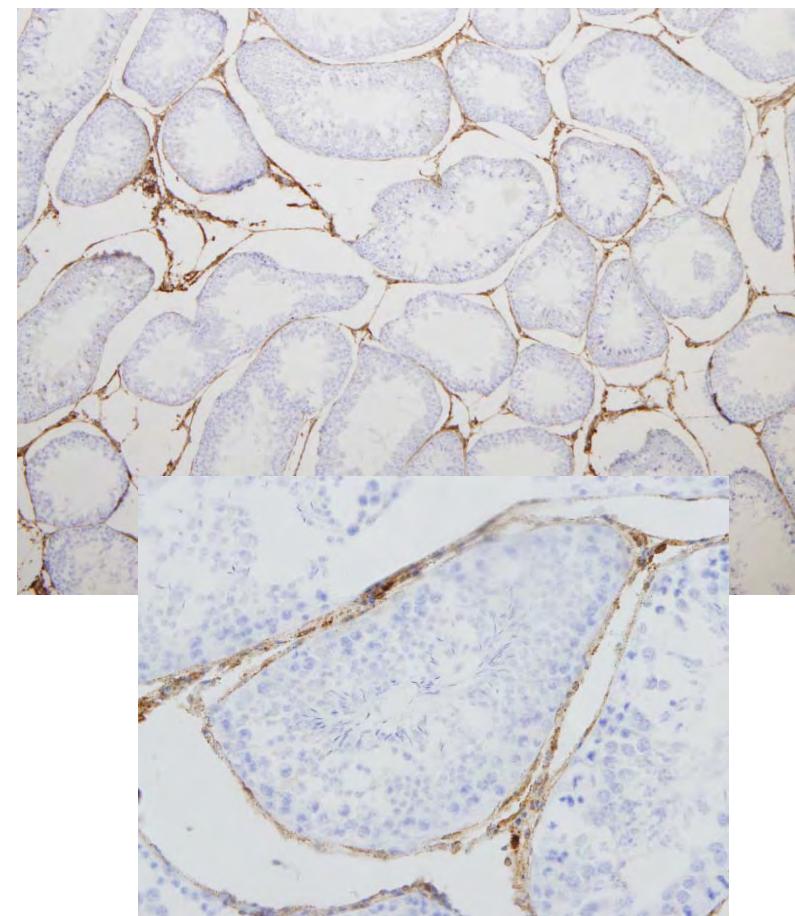
Espressione di interferon-induced MX-1 protein nel testicolo di montoni infettati con BTV-1_{IT2006} e BVT-1_{IT2013}

MX-1

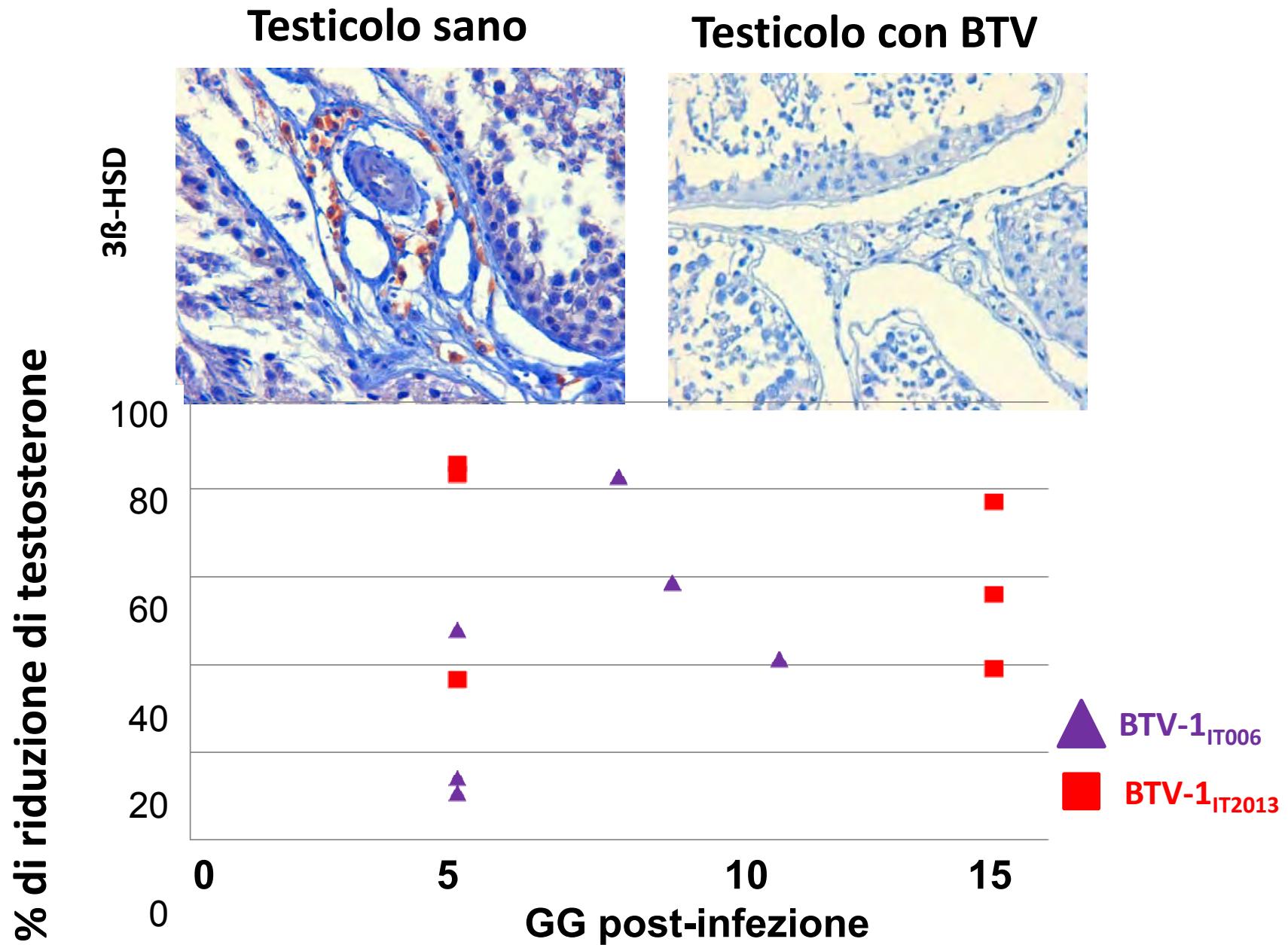
Testicolo sano



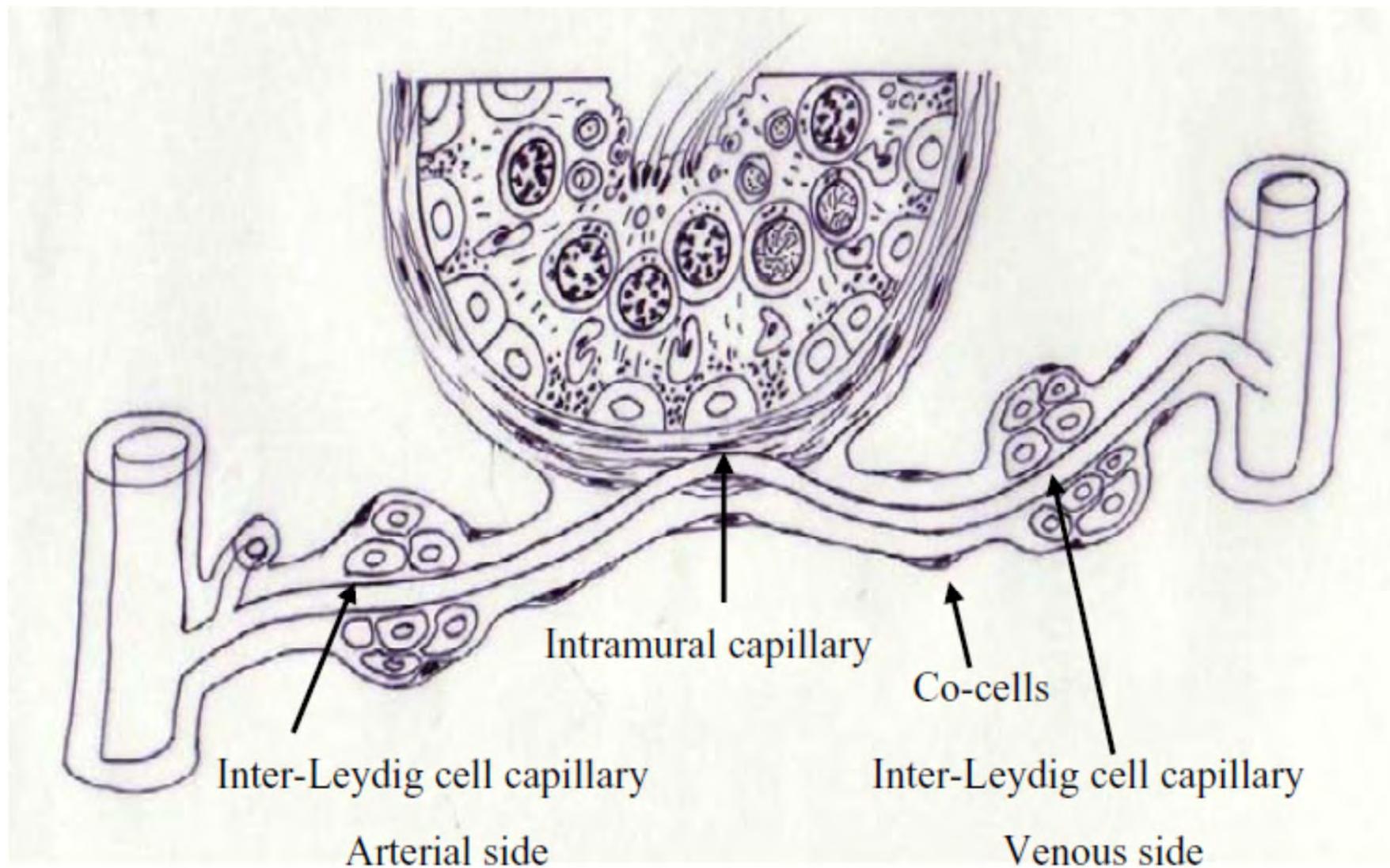
Testicolo con BTV



Espressione di 3β -HSD nel testicolo



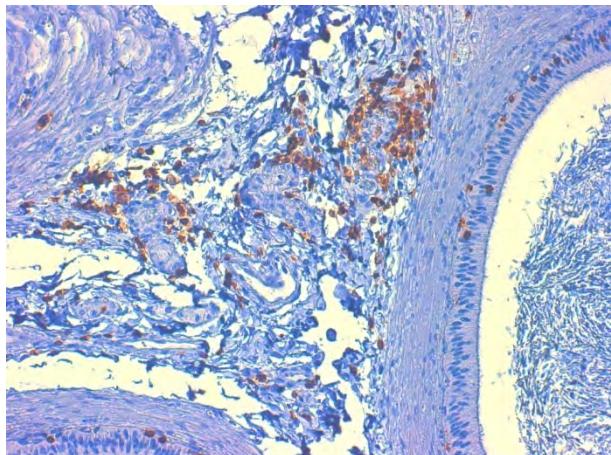
Micro circolazione Leydig - tubuli seminiferi.



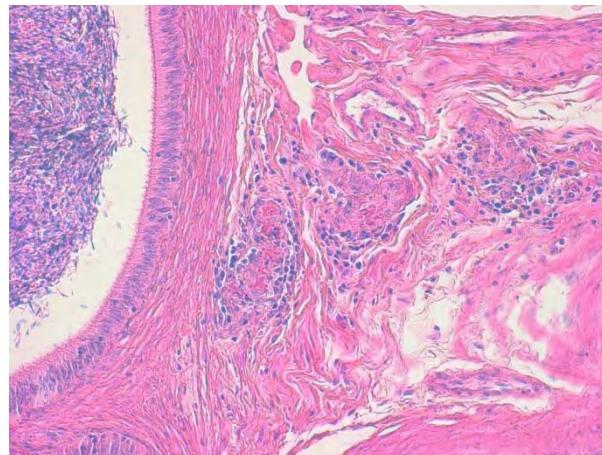
LINFOCITI T CD +

Epididimo

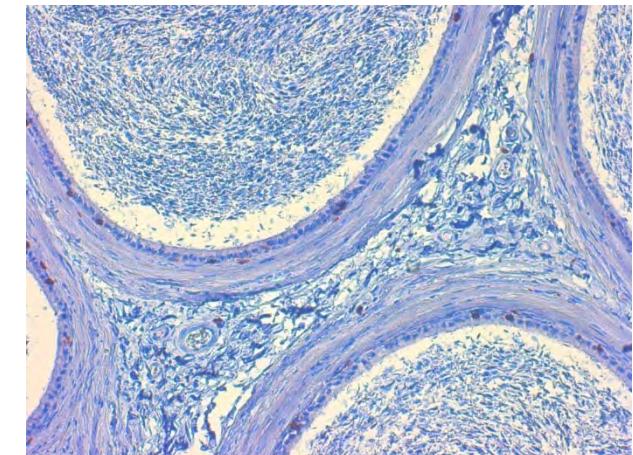
BTV



BTV

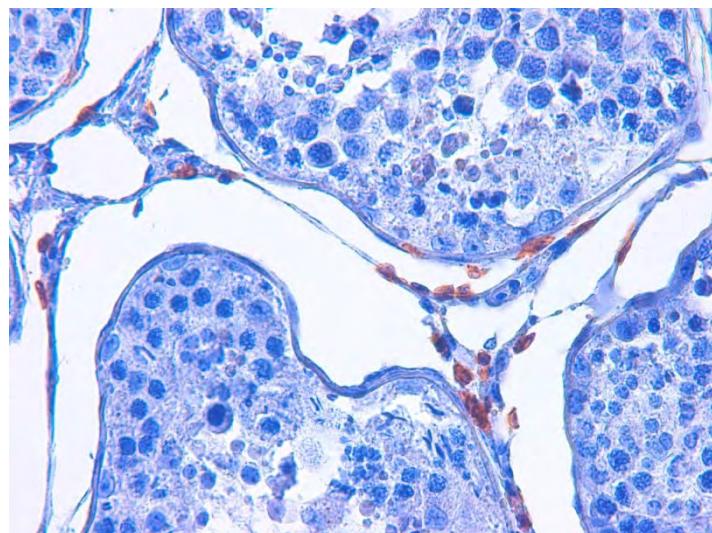


Controllo

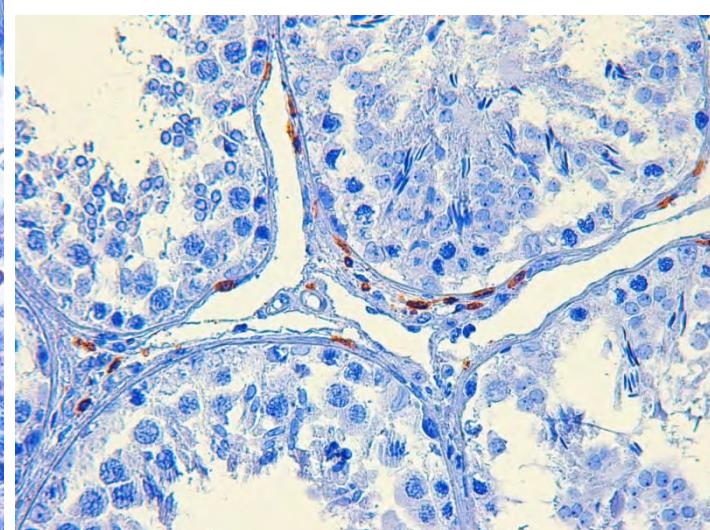


Testicolo

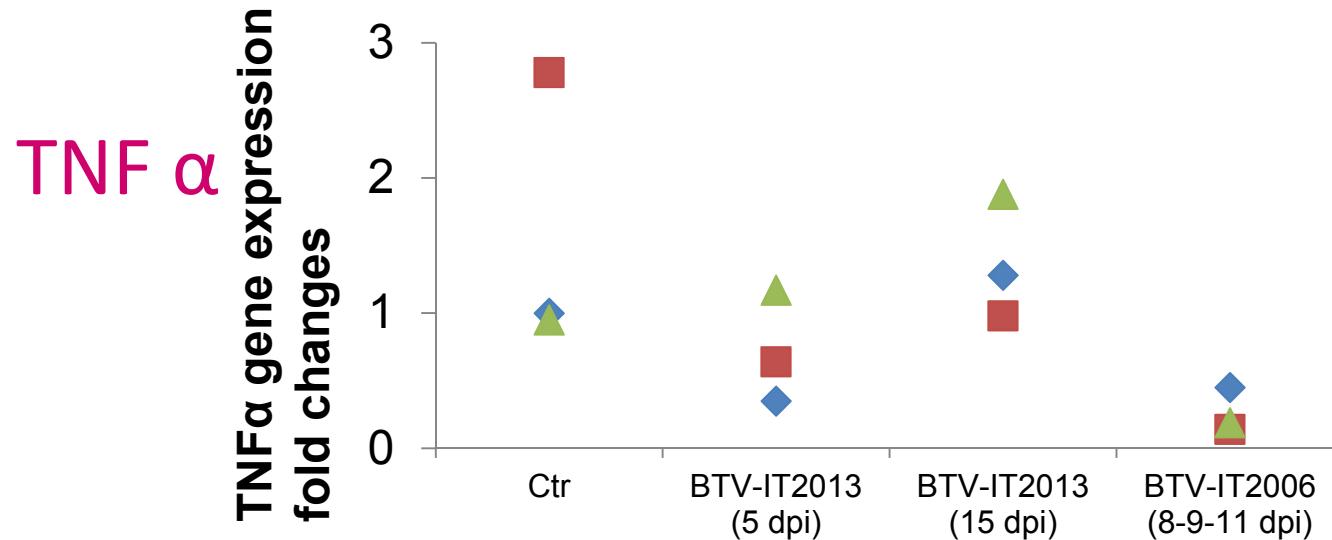
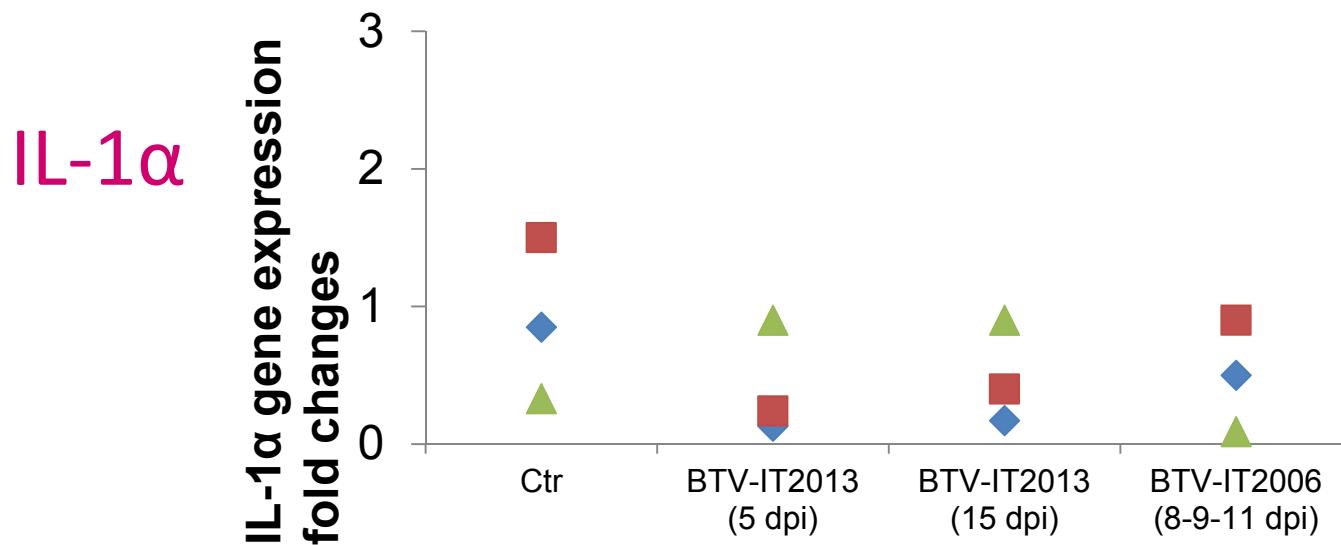
Controllo



BTV



RT RT-PCR gene expression per TNF α e IL1 α nel testicolo di ariete infettato con BTV-1_{IT2013} e BTV-1_{IT2006}



Considerazioni

Infezioni da BTV con differenti isolati dello stesso sierotipo possono risultare in quadri clinici significativamente dissimili in termini di gravità dei sintomi e mortalità.

La differente virulenza tra sierotipi e/o isolati di BTV è in parte dovuta ad una diversa capacità di compromettere la risposta immunitaria acquisita dell'ospite, indicendo una temporanea disorganizzazione dell'architettura follicolare dei linfonodi periferici (e non ?).

Inoltre, una differente virulenza si basa sulla capacità dell'isolato di BTV di danneggiare a livello cellulare la funzionalità dell'organo, anche a seguito della risposta innata dell'ospite *via interferone* (??).

GRAZIE PER LA VOstra ATTENZIONE



PHOTO BY F DEMONTIS