



# ***Un overview sulla circolazione di BDV- 8 in Italia nell'interfaccia domestici/selvatici***


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## BRIEF REPORT

## A new genotype of border disease virus with implications for molecular diagnostics

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### Rapid Communication

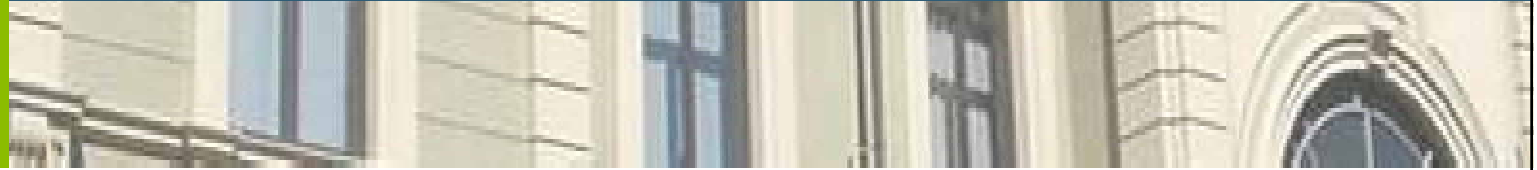
#### Evidence of circulation of the novel Border Disease Virus genotype 8 in chamois.

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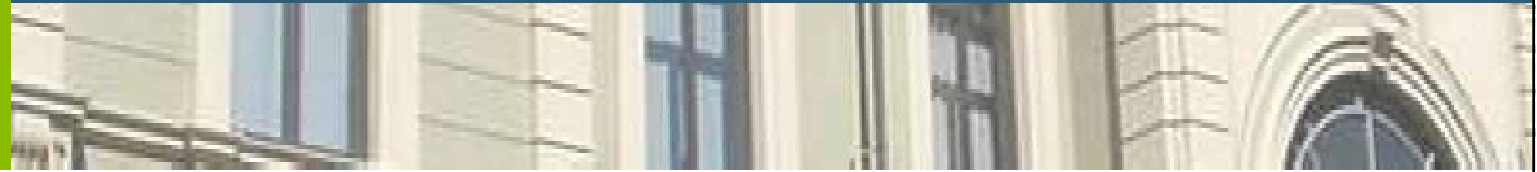
(Accepted ; Archives of Virology, *In press*)



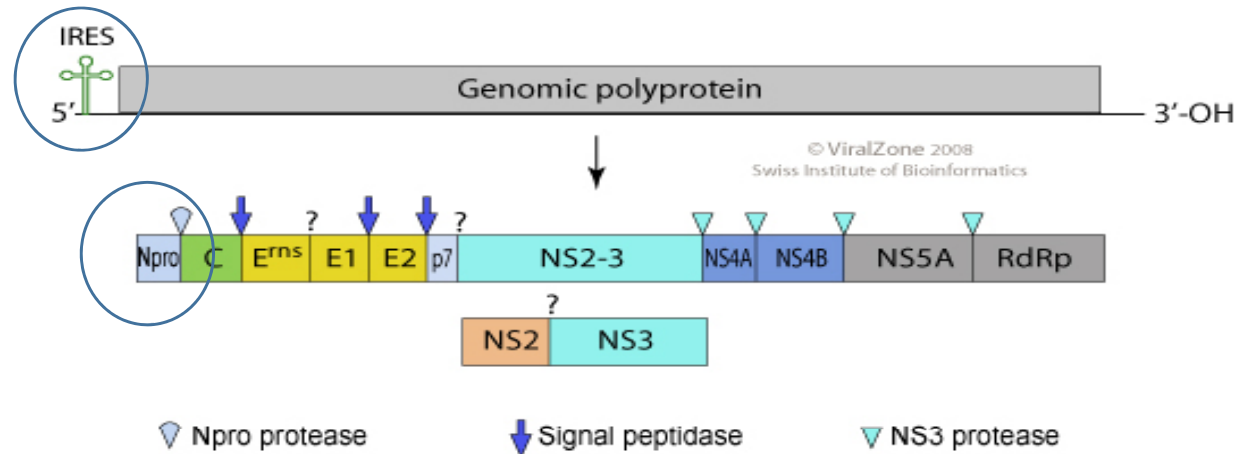
[-] Family: <i>Flaviviridae</i>	(4 Genera) ◀ history ▶
[-] Genus: <i>Flavivirus</i>	(53 Species) ◀ history ▶
[-] Genus: <i>Hepacivirus</i>	(1 Species) ◀ history ▶
[-] Genus: <i>Pegivirus</i>	(2 Species) ◀ history ▶
[-] Genus: <i>Pestivirus</i>	(4 Species) ◀ history ▶
Species: <i>Border disease virus</i> ←	◀ history ▶
★ Species: <i>Bovine viral diarrhoea virus 1</i>	◀ history ▶
Species: <i>Bovine viral diarrhoea virus 2</i>	◀ history ▶
Species: <i>Classical swine fever virus</i>	◀ history ▶

- Aborti;
- Infertilità;
- Ritardo nella crescita;
- Soggetti «shaker»;
- Immunosoppressione.

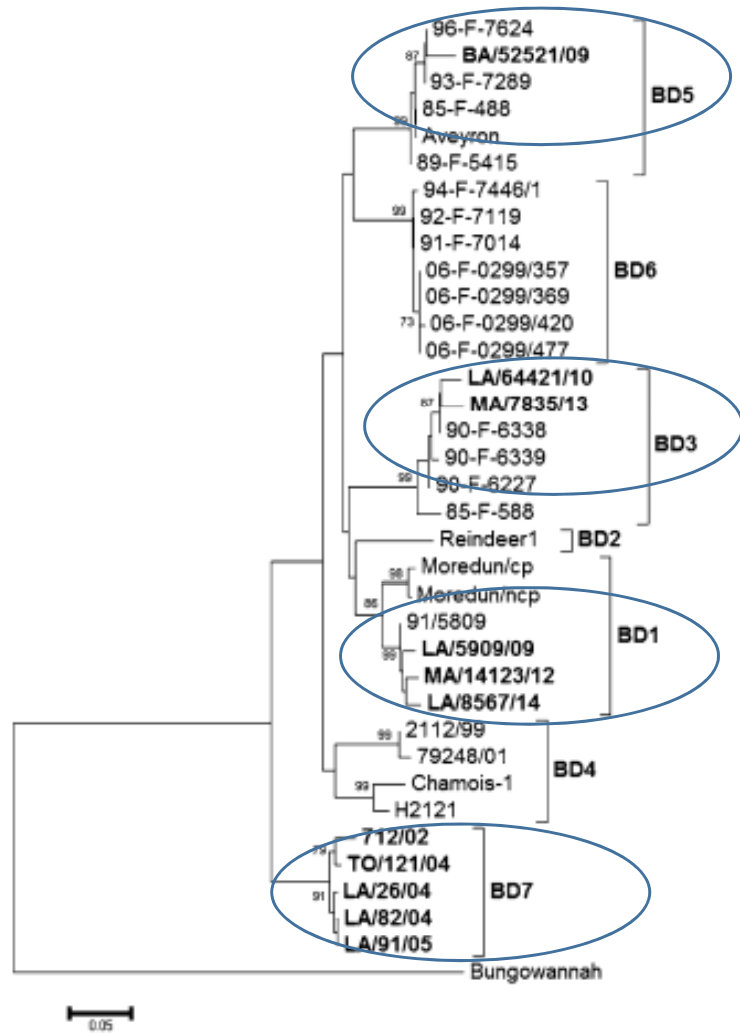
INFEZIONE TRANSPLACENTARE = animali persistentemente infetti (PI) o immunotolleranti



- c.a. 12 Kb
- 4 ST vs 8 NS



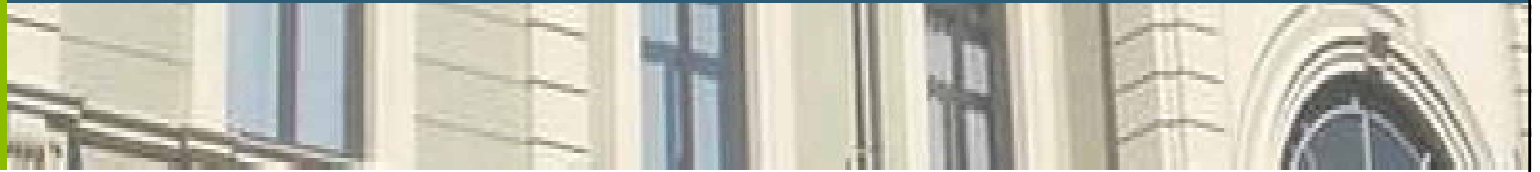
- BDV-1: (1a e 1 b) include isolati ovini USA, UK, Nuova Zelanda;
- BDV-2: ruminanti in Germania;
- BDV-3: ovini in Svizzera e Italia;
- BDV-4: camosci dei pirenei (*Rupicapra pyrenaica*);
- BDV – 5,6: identificato in Francia;
- BDV- 7: isolati caprini e ovini sul territorio italiano.



Lazio, Marche, Toscana e Basilicata  
arco temporale 2002 - 2014

**BDV-1; BDV-3; BDV-5; BDV -7**

*Giammarioli et al., 2015 Virus genes*



**BDV-8**

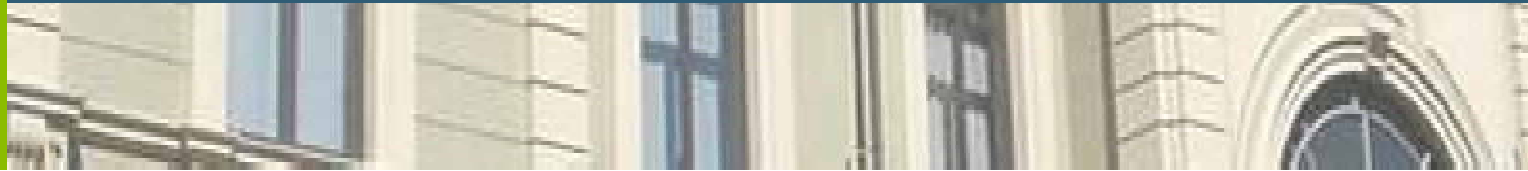


Allevamento misto (24 bovini / 20 caprini) della provincia di Torino. L'esame autoptico evidenziava:

- ulcere;
- erosioni abomasali.

Camoscio alpino (*Rupicapra rupicapra*): pervenuto presso Ce.R.Ma.S..

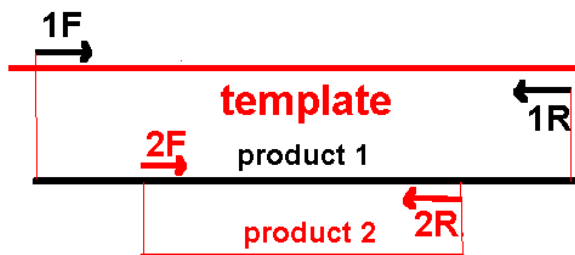
- focolai multipli di broncopolmonite;
- marmorizzazione polmonare;
- aderenze pleuriche interlobari



**Border disease virus** → Vilcek et al., 1994

TARGET	STEP	PRIMERS	SEQUENZA 5'-3'	LENGTH (bp)
5'UTR	1st	BE first 05	CAT GCCCTTAGTAGGACTAGC	288
		B2 first 03	TCAACTCCATGT-GCCATGTAC	
	2nd	PBD1 second 05	TCGTGGTGAGATCCCTGAG	225
		PBD2 second 03	GCAGAGATTTTTTATACTAGCC TATRC	

**NESTED PRIMER PCR:**



BE – B2



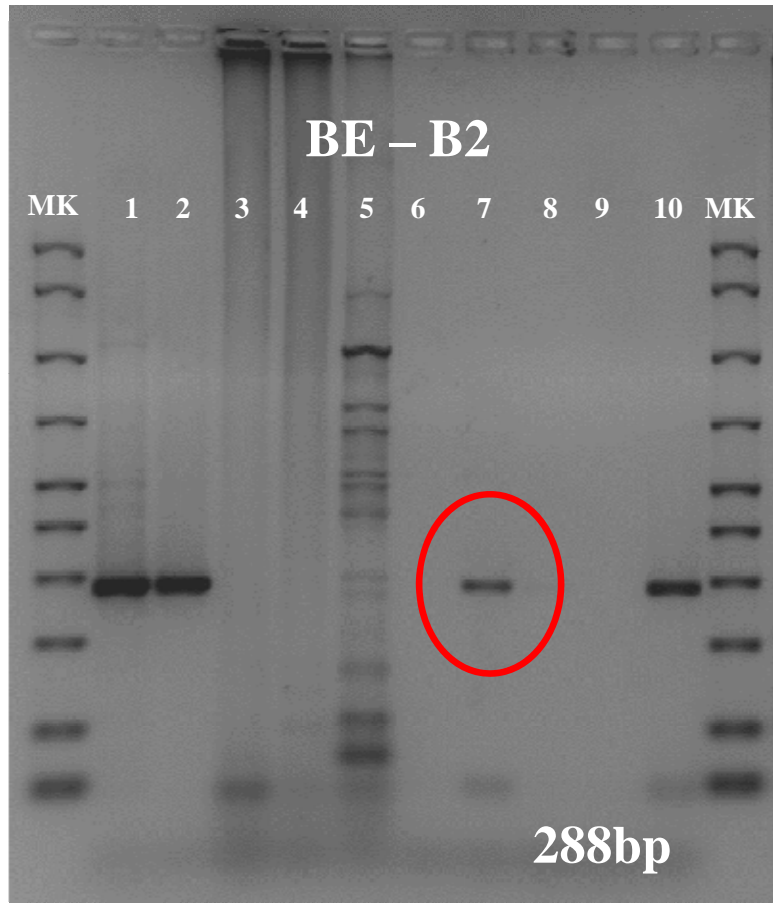
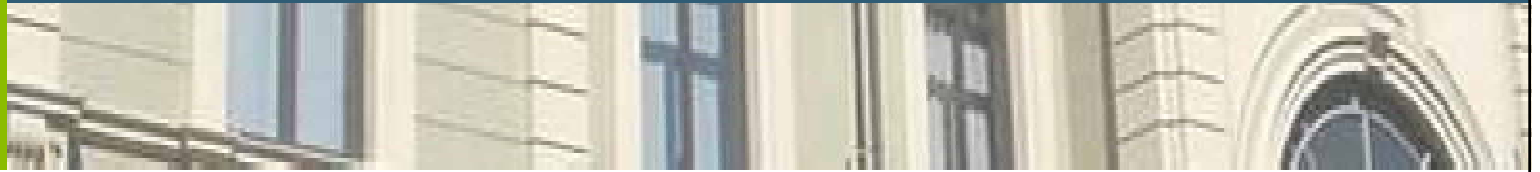
Panpestivirus

PBD1 – PBD2

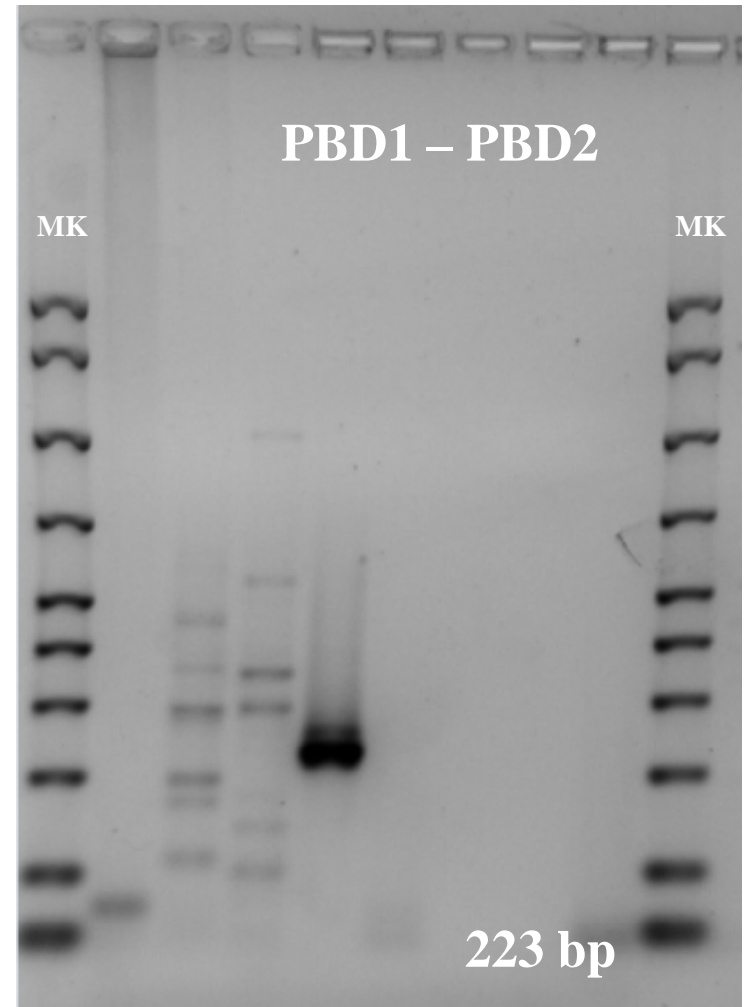


Specifici BDV





7: milza capra  
8: bEST  
9: bPCR  
10: C+ BVDV



..si otteneva...

No amplificazione





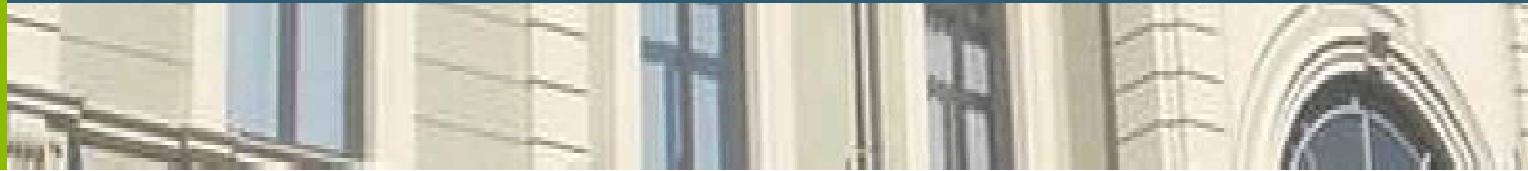
~~**PBD2 GCAGAGATTTTATACTAGCCTATRC**~~

*mismatches* tra 5'UTR virale ed estremità 3' del reverse primer (PDB2) specifico per BDV: mutazioni in siti comuni, ma divergenti tra i due ceppi (triplette ATA e ACA, rispettivamente).

(B)

	PDB2				
	220	230	240	250	260
More dun cp BDV1 U65022	CAGAGGCCCA	-	CGTATAGGCTAGTATA	AAAAA	TCTCTGCT
91.5809 BDV1 AF026768	.....	..C	.....	.....	.....
Rudolph BDV2 AB122086	.....	..C	..A	.....	.....
Reindeer1 BDV2 NC003677	.....	..C	..A	.....	.....
85F488 BDV3 EF693985	.....	..C	..A	.....	.....
90F6338 BDV3 EF693991	.....	..C	.....	.....	.....
90F6227 BDV3 EF693989	.....	..C	.....	.....	.....
Chamois BDV4 AY738080	.....	..T	..A	.....	..A
C121 BDV4 DQ275625	.....	..T	.....	.....	.....
2112/99 BDV4 AY159513	.....	..TC	.....	.....	.....
79248/01 BDV4 AY159515	.....	..TC	.....	.....	.....
96F7624 BDV5 EF693998	.....	..C	..A	.....	.....
AV BDV5 EF693984	.....	..C	..A	.....	.....
89F5415 BDV5 EF693988	.....	..C	..A	.....	.....
91F7014 BDV6 EF693993	.....	..C	.....	.....	.....
06F029960.477 BDV6 EF694003	.....	..C	.....	.....	.....
94F7446.1 BDV6 EF693996	.....	.....	.....	..A	.....
06F0299.369 BDV6 EF694001	.....	..C	.....	.....	.....
712.02 BDV7 AJ829444	.....	..T	..C	.....	..C
TO.121.04 BDV7 AM900848	.....	..T	..C	.....	.....
LA.82.04 BDV7 FM163383	.....	..T	..C	..C	.....
LA.26.04 BDV7 FM163382	.....	..T	..C	..C	.....
LA.91.05 BDV7 FM163381	.....	..T	..C	..C	.....
103761.2.1 BDV8 Torino	.....	..C	..ATA	.....	.....
Burdur/05 Turkey AM418428	.....	..TA	.....	.....	.....
Aydin/04 Turkey AM418427	.....	..TG	..C	.....	.....
SN2T Tunisian AF461996	.....	..A	.....	..TAG	.....
BM01.3 Tunisian AY453630	.....	..A	.....	..TAG	.....
SN3G Tunisian AY583306	.....	..A	.....	..TAG	..AA

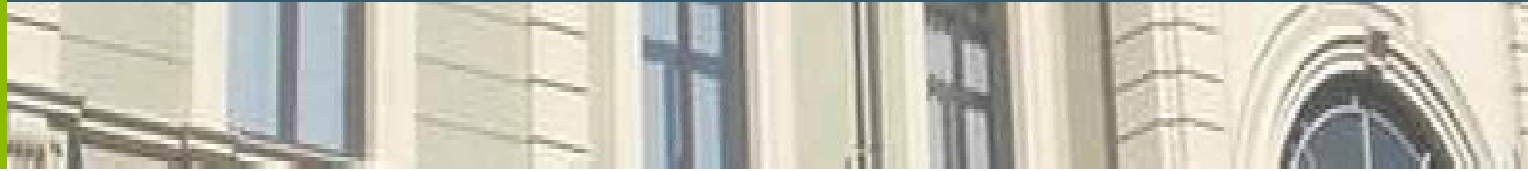
Tali mutazioni, seppur minime hanno causato un non corretto funzionamento della coppia di primers interna e il fallimento de protocollo molecolare



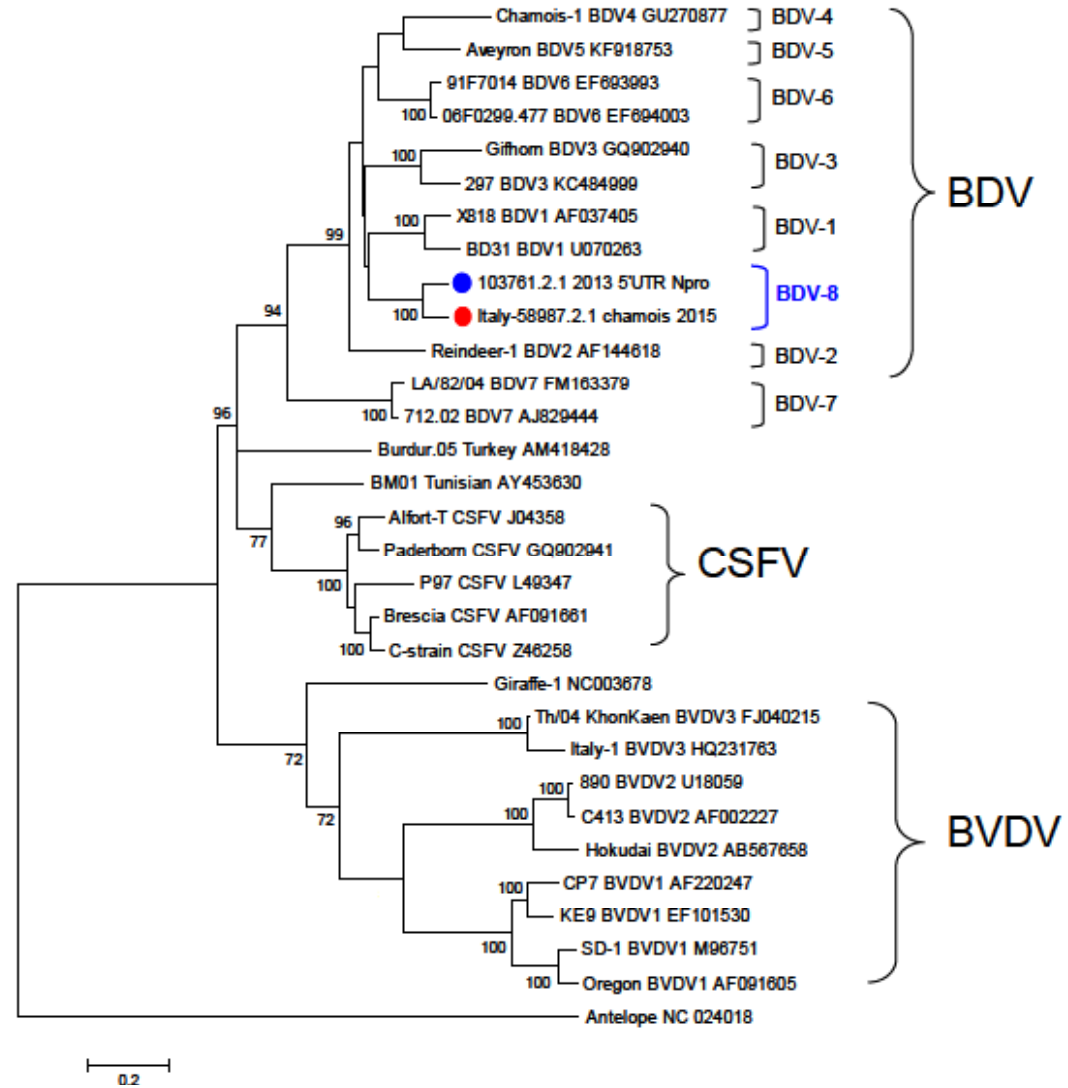
77.8%-89.7% 5'UTR

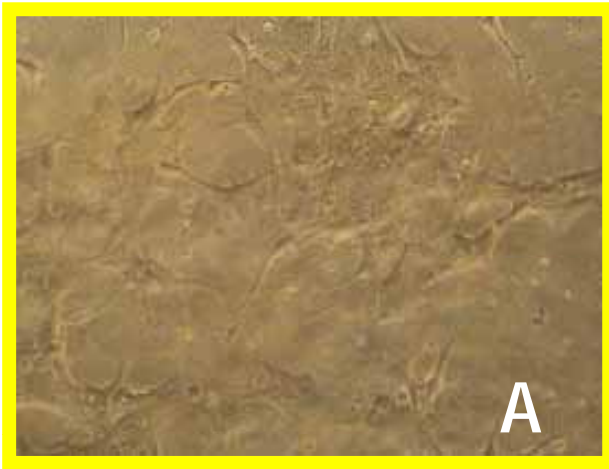
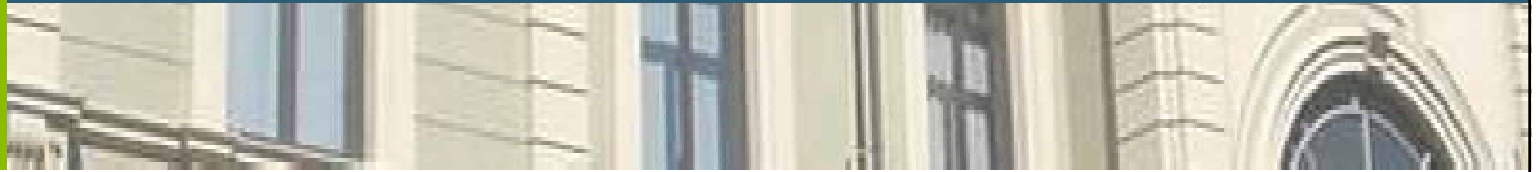
66.5%-78.4% Npro

	Torino-103761 BDV-8	Morehun BDV-1 U65022	Reindeer BDV-2 NC003677	90F6338 BDV-3 EF693991	Chamois BDV-4 AY738080	AV BDV-5 EF693984	91F7014 BDV-6 EF693993	TO.121.04 BDV-7 AM900848	BM01.3 Tunisian AY453630	05 Turkey AM418428	NADL BVDV-1 M31182	890 BVDV-2 U18059	Giraffe AB040131	Pronghorn AY781152	Brescia CSFV M31768	5'-UTR
<b>Torino-103761 BDV-8</b>		88.5	88.1	86.4	84.8	89.7	87.7	83.1	77.8	78.2	72.4	68.3	70.8	63.8	81.9	<b>Torino-103761 BDV-8</b>
X818 BDV-1 AF037405	77.0		88.9	90.1	84.4	88.5	89.7	83.5	82.3	82.7	71.2	66.7	70.0	64.2	82.3	Morehun BDV-1 U65022
Reindeer BDV-2 NC003677	71.3	72.0		87.7	83.5	87.7	87.2	82.7	81.1	81.1	72.8	66.7	72.4	60.9	81.9	Reindeer BDV-2 NC003677
90F6338 BDV-3 EF693969	73.9	72.2	72.2		83.1	86.4	86.8	82.7	77.4	79.8	73.3	69.1	71.2	63.0	81.5	90F6338 BDV-3 EF693991
Chamois BDV-4 GU270877	73.4	73.4	72.0	72.7		88.9	86.4	83.5	77.4	76.1	71.6	68.7	72.0	62.1	80.2	Chamois BDV-4 AY738080
AV BDV-5 EF693962	74.1	76.2	71.5	74.8	75.1		90.1	82.7	77.4	76.5	72.0	68.7	70.8	60.5	78.2	AV BDV-5 EF693984
91F7014 BDV-6 EF693971	78.4	75.5	72.2	74.8	73.2	76.2		82.7	79.8	77.4	72.4	68.3	72.8	60.5	80.2	91F7014 BDV-6 EF693993
TO.121.04 BDV-7 AM900847	71.7	69.1	71.7	71.3	73.4	69.8	70.8		70.5	70.1	63.9	65.3	65.6	63.0	68.4	TO.121.04 BDV-7 AM900848
BM01.3 Tunisian AY453629	69.8	70.3	69.8	70.3	71.5	71.0	67.2	79.4		71.3	72.8	71.2	80.2	65.4	82.3	BM01.3 Tunisian AY453630
05 Turkey EU930015	66.5	68.2	66.5	65.6	67.9	67.0	66.5	79.8	80.2		74.1	68.7	72.4	63.0	82.7	05 Turkey AM418428
BVDV-1 Oregon AF091605	65.8	64.6	66.7	62.9	66.5	63.4	64.4	72.0	63.2	60.3		66.0	69.5	63.0	66.3	NADL BVDV-1 M31182
Hokudai BVDV-2 AB567658	65.6	64.6	66.7	63.7	64.1	66.3	64.1	65.8	65.8	67.0	74.9		70.0	62.1	67.7	890 BVDV-2 U18059
Giraffe AF144617	65.8	63.2	62.9	63.9	66.7	67.0	73.2	74.1	67.2	63.9	66.7	64.6		59.7	65.8	Giraffe AB040131
Pronghorn AY781152	58.9	61.8	58.9	59.1	58.4	59.6	62.0	62.7	62.2	62.9	62.2	62.9	59.1		62.8	Pronghorn AY781152
Brescia CSFV AF091661	69.1	68.4	69.4	66.5	69.1	67.5	67.2	80.2	75.5	69.8	71.2	68.3	74.5	63.0		Brescia CSFV M31768
<b>Npro</b>	<b>Torino-103761 BDV-8</b>	<b>Morehun BDV-1 U65022</b>	<b>Reindeer BDV-2 NC003677</b>	<b>90F6338 BDV-3 EF693991</b>	<b>Chamois BDV-4 AY738080</b>	<b>AV BDV-5 EF693984</b>	<b>91F7014 BDV-6 EF693993</b>	<b>TO.121.04 BDV-7 AM900848</b>	<b>BM01.3 Tunisian AY453630</b>	<b>05 Turkey AM418428</b>	<b>NADL BVDV-1 M31182</b>	<b>890 BVDV-2 U18059</b>	<b>Giraffe AB040131</b>	<b>Pronghorn AY781152</b>	<b>Brescia CSFV M31768</b>	



- Isolato caprino
- Isolato camoscio alpino 2015
- ● Identità 93.6% e 92.3%





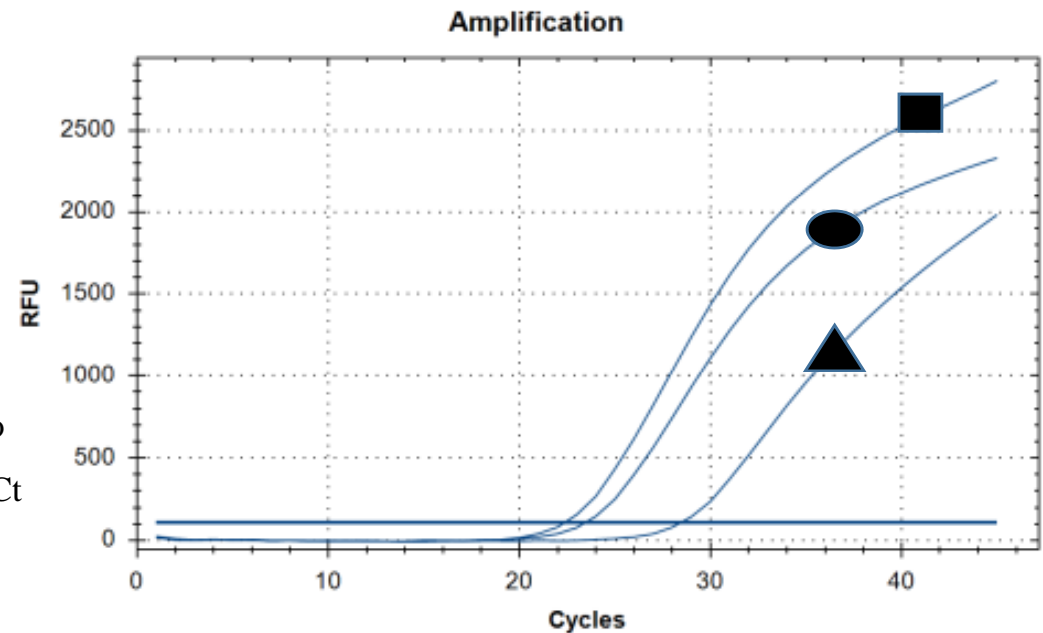
**Fig.1**

A: Modificazione del monostrato cellulare SFTR osservato al I passaggio (4x) polmone di camoscio  
B: monostrato SFTR non infetto



**Fig. 2**

La Real-time RT-PCR effettuata su surnatante di SFTR al I passaggio (freccia), II (cerchio) e III (quadrato) ha mostrato un aumento in cT (Ct values 28.03, 23.06, and 22.02, respectively).





milza

polmone

pre- trattati con Ear notch lysis buffer

## IDEXX BVDV Ag/Serum Plus Test

E<sup>rn</sup>s (gp44-48);

Erns ELISA		
1	1,811	1-5= BD3 cute auricolare capra
2	1,802	2-6= BD3 milza capra
3	3,077	3-7= BD8 polmone camoscio
4	2,705	4-8= BD8 milza camoscio
5	1,775	
6	1,698	
7	2,986	
8	2,659	



# FOCUS

DIAGNOSTICI



Fallimento protocollo PCR (Vilcek *et al.*, 1994)  
largamente utilizzato presso i laboratori diagnostici



Primaria importanza sviluppo e validazione di una  
metodica biomolecolare con primers generici per  
tutti i genotipi BDV



# FOCUS

EPIDEMIOLOGICI →

2014



2015



pascolo;  
monticazione;  
biosicurezza.

Epidemiological study of border disease virus infection  
in Southern chamois (*Rupicapra pyrenaica*) after an  
outbreak of disease in the Pyrenees (NE Spain)

Ignasi Marco<sup>a,\*</sup>, Rosa Rosell<sup>b,c</sup>, Oscar Cabezón<sup>a</sup>, Gregorio Mentaberre<sup>a</sup>,  
Encarna Casas<sup>a</sup>, Roser Velarde<sup>a</sup>, Jorge Ramón López-Olvera<sup>d</sup>,  
Ana H... Lavín<sup>a</sup>

**BDV-4**

↓  
**40%-45%**

BDV-8

*M. haemolytica*



Patogeno sinergico / emergente





**GRAZIE PER L'ATTENZIONE**