



Sistemi Diagnostici Point of Care per l'identificazione rapida di *Poxvirus* zoonosici

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Point-of-care (POC) testing are defined as medical testing at or near the site of patient care

Veterinary diagnostics is a key tool in the prevention and control of infectious diseases in animals. POC4PETS aims at delivering an innovative set of technologies for sample preparation and enrichment for point of care (POC) diagnosis of pet diseases.

KEY OBJECTIVES

POC4PETS focuses on emerging, endemic and zoonotic diseases, currently lacking reliable diagnostic tools. The research activities aim at identifying the agents via different **nucleic acid detection technologies** such as: on site PCR, isothermal amplification and array probing.



Papillomaviruses



Leishmania



Zoonotic poxviruses

SELECTED DISEASES



Feline and canine parvovirus - feline respiratory viruses

KEY FACTS

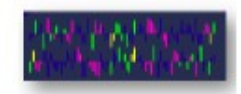
Research for the benefit of SMEs
 Project: **FP7 -7 -SME - 2012 - 1**
 Grant agreement No: **315653**
 Start date: 01/09/2012
 End date: 31/08/2014
Project Coordinator:
 Alessandra Scagliarini
Project Manager:
 Alessandra Baccigotti

SKULDTECH	ICN2
vetgenomics	ennebi



THE CONSORTIUM

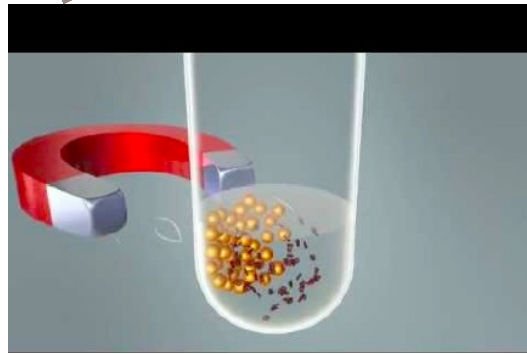
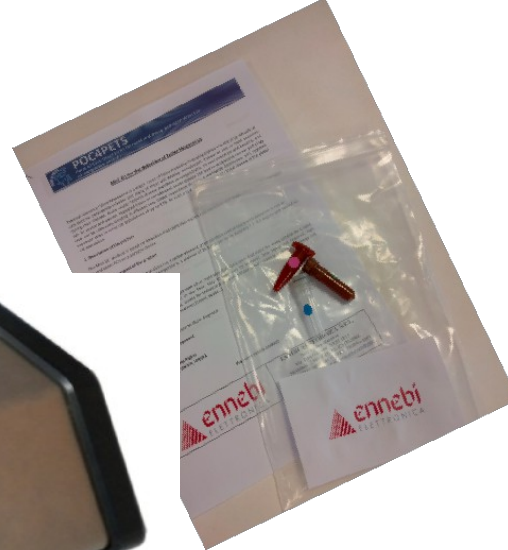
The consortium involves RTD performers with expertise in virology, microbiology, parasitology, chemistry, nanotechnology, molecular biology and SMEs involved in veterinary diagnostics manufacturing, laser technology and veterinary



POC4PETS will provide the SMEs with new tools and effective detection systems characterised by the potential of a high market acceptance enhancing their competitiveness and at the same time contributing to reach the CAHP 2007-2013 objectives.

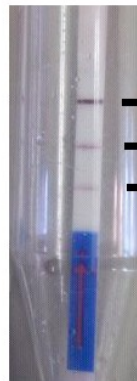
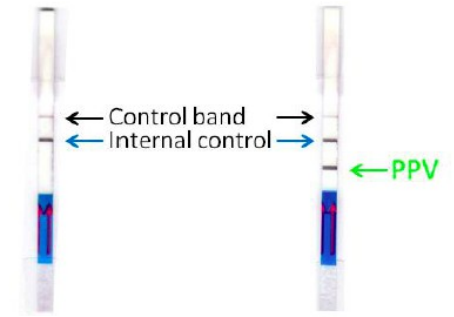
FROM GAP ANALYSIS TO SOLUTIONS





Non infected

PPV infected



→ Control line
→ Endogenous control line
→ Leishmania Test line

Commercial diagnostic kits available worldwide

No universal diagnostic test in use when available, is mainly performed at academic institutions or in public health reference laboratories.

GAP: rapid (validated), point-of-care tests are not available.

Commercial diagnostic kits available in Europe

No universal diagnostic test in use when available, is mainly performed at academic institutions or in public health reference laboratories.

GAP: rapid (validated), point-of-care tests are not available.

Diagnostic kits validated by International, European or National Standards

None.

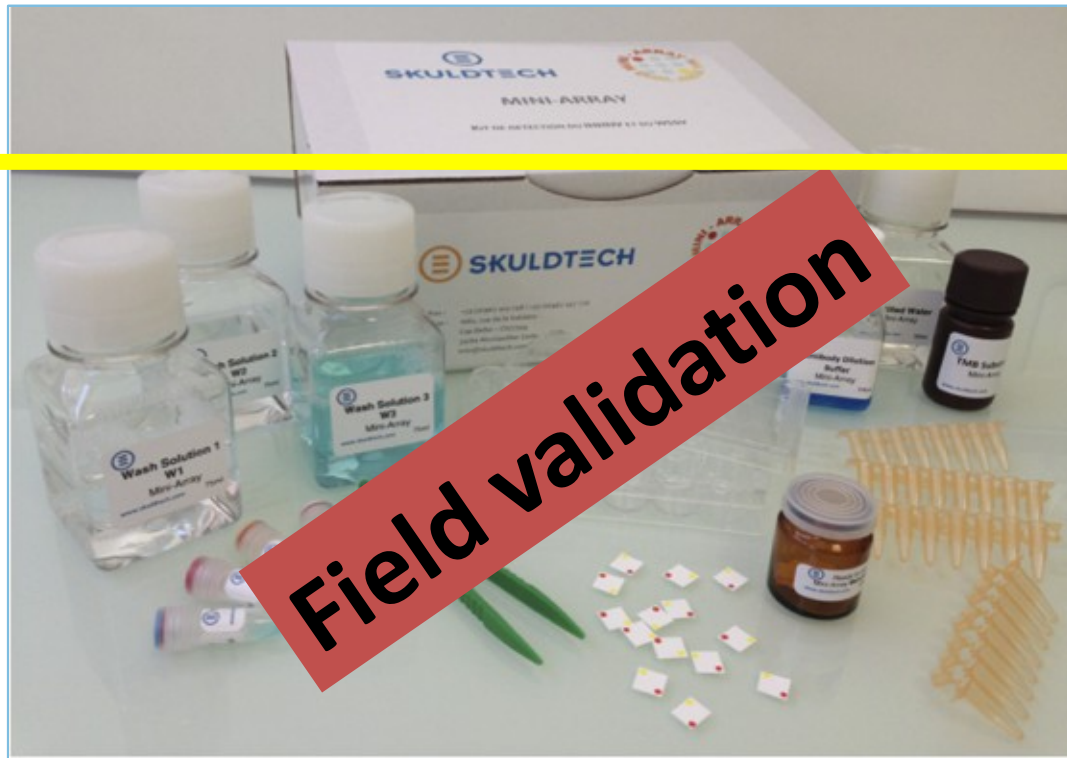
Diagnostic method(s) described by International, European or National standards

None.

Commercial potential for diagnostic kits in Europe

Recently, possibly due to the increase in severe outbreaks of disease, labs across Europe, the US, Japan, India and South Africa have shown an interest in the parapoxviruses. In most instances this has been to confirm diagnosis of disease outbreaks, thereby reducing the incidence of misdiagnosis. At present there are no routine diagnostic tests in use for poxviruses and this can lead to misdiagnosis with other pathogens causing vesicular disease in ruminants. Practical problems emerged during the 2001 pan-asiatic type O FMD outbreak in the UK because of the difficulty of diagnosing FMD in sheep and cattle. Nowadays this can be again a cause of concern due to the recent spread of Bluetongue virus in Northern Europe and vaccinia-like outbreaks in cattle in several countries.

GAP: these zoonotic infections share clinical manifestations and exposure risks with other, potentially life-threatening zoonoses (e.g., cutaneous anthrax) and are likely under-recognized because of a lack of clinical suspicion and widely available diagnostics.



In collaboration with Area di diagnostica virologica IZS Sicilia



AIM

Genomic characterisation of
epitheliotropic viruses
identified in proliferative lesions
clinically defined as "Papillomas"
in domestic ruminants

Papillomaviridae

- ds DNA naked viruses

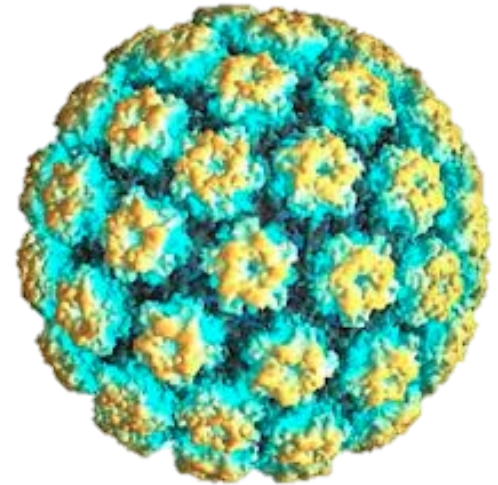
- 55 nm ø
- Circular genome ~ 8 kb

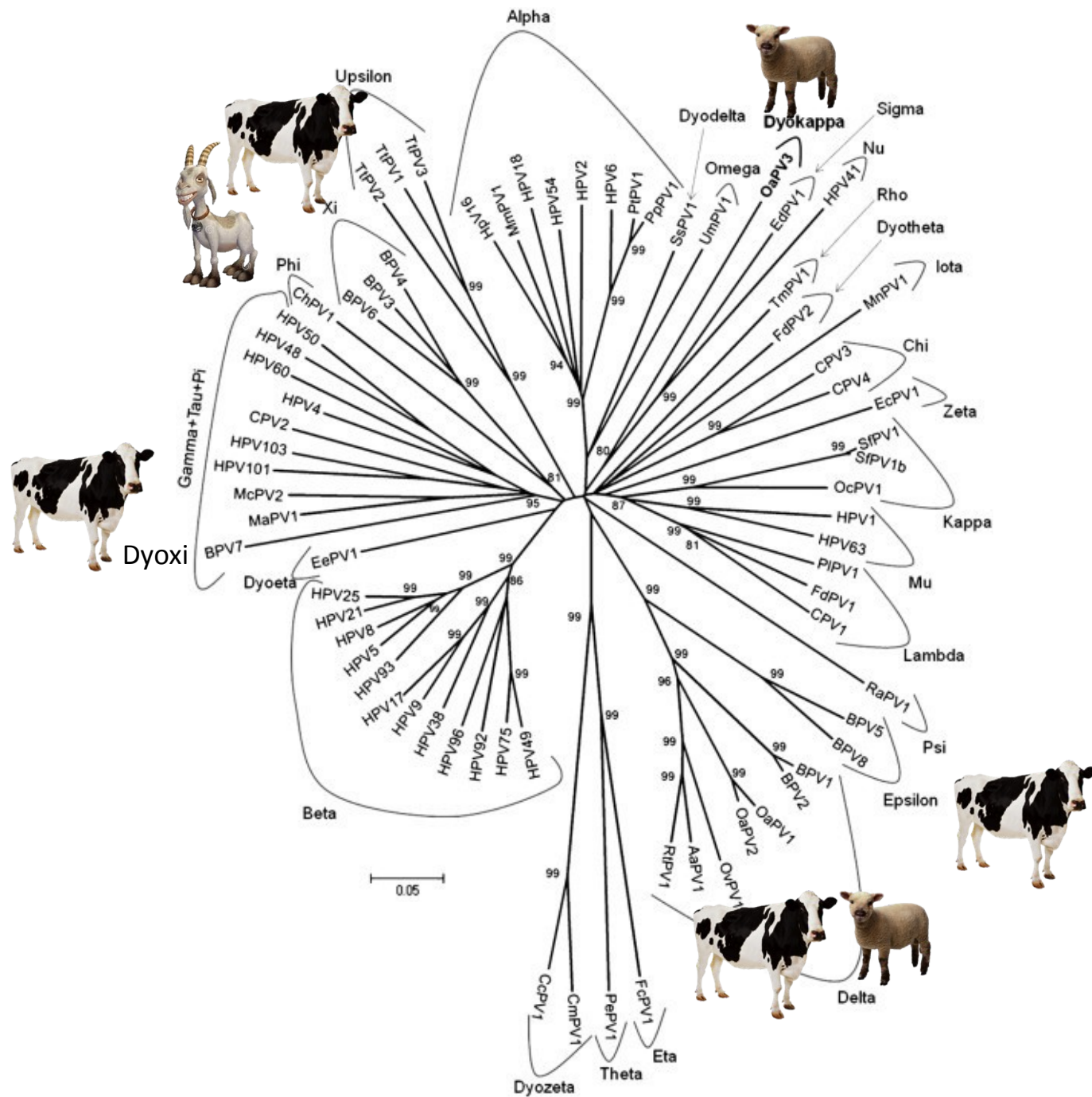
- Wide distribution among animals

- Highly species-specific

- Tropism for squamous epithelia

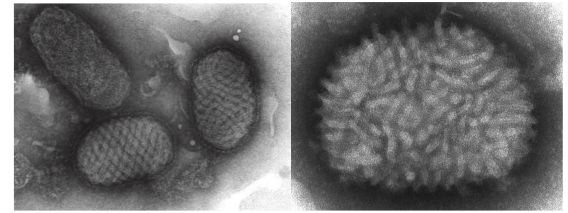
- Nuclear replication





Poxviridae

Chordopoxvirinae



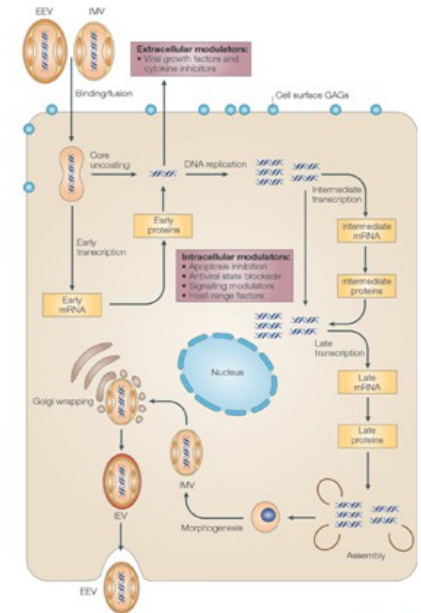
➤ ds DNA viruses with or without envelope

- Linear genome 130 - 300 kb
- 260-350 nm length, 160-250 nm width

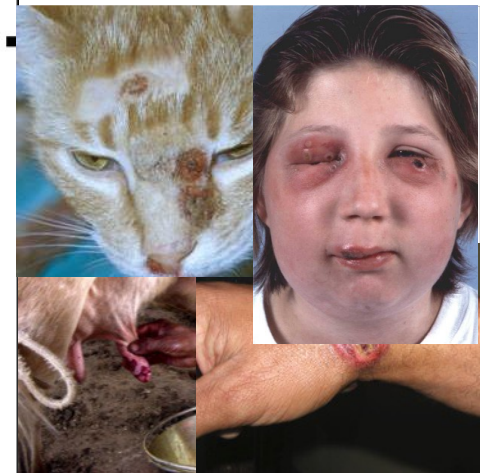
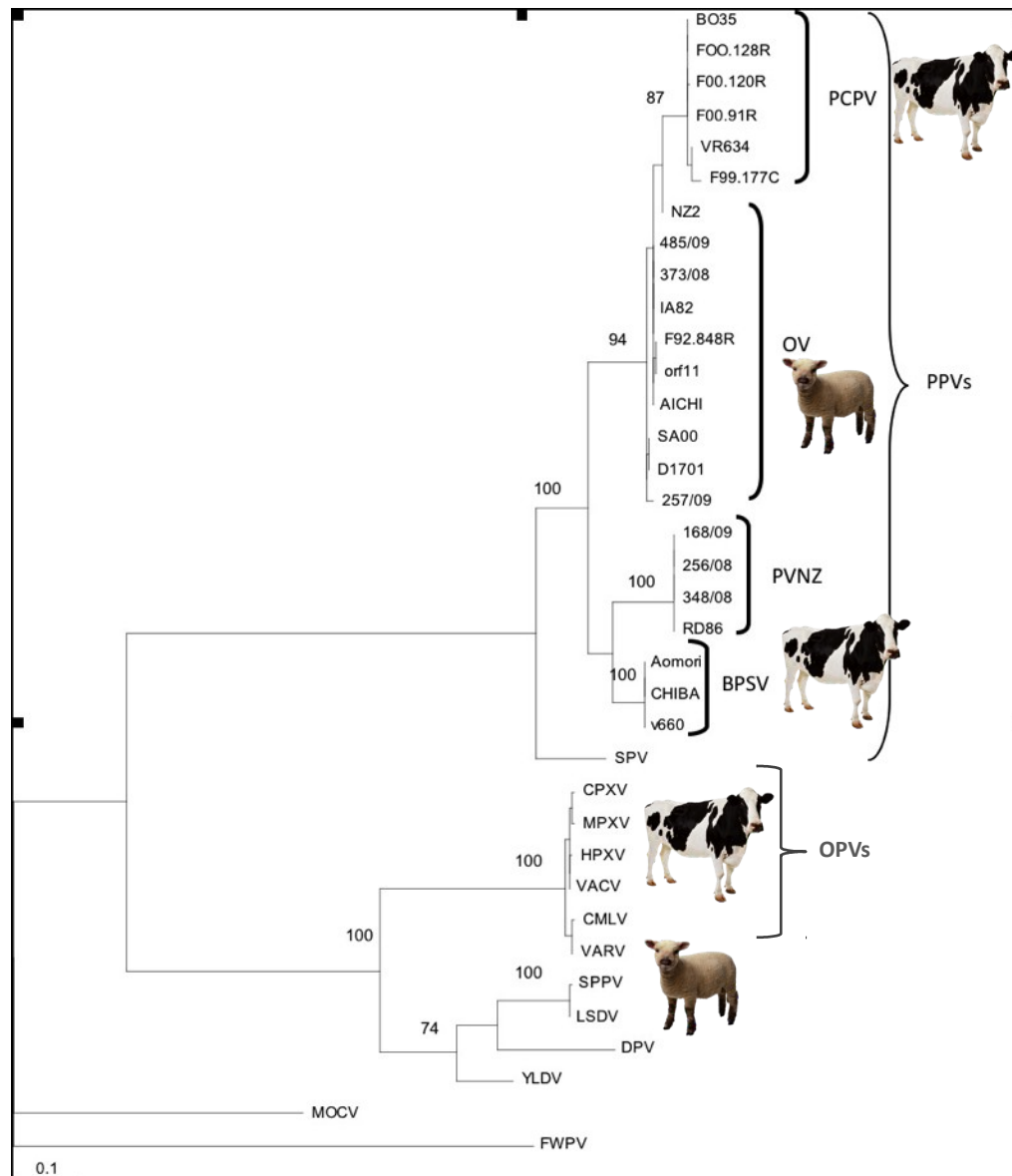
➤ Wide distribution among animals

- Highly species-specific
- frequently ZOONOTIC

- TROPISM for epithelial cell of the skin and mucosa
- Replication in the cytoplasm
- Encode their own DNA dependent RNA polymerase



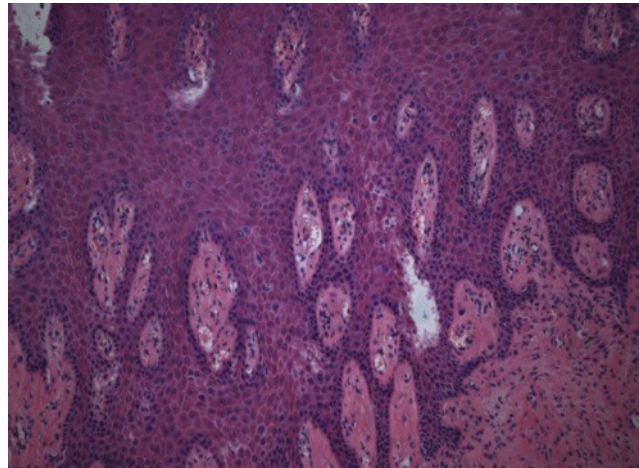
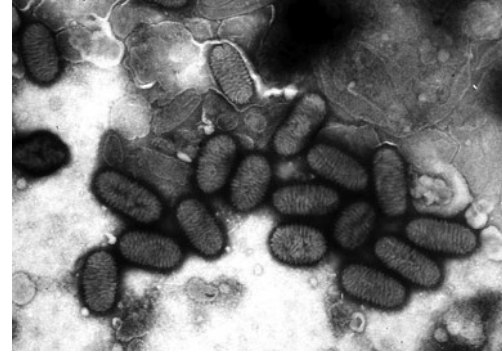
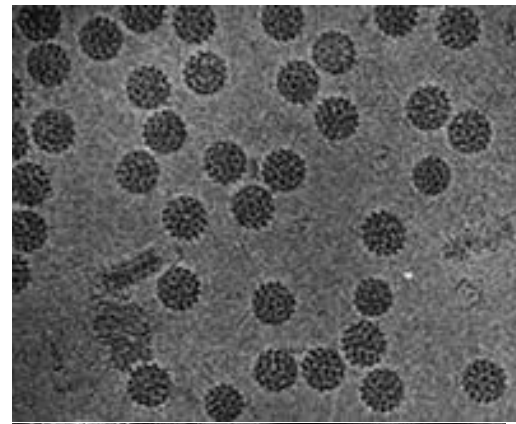
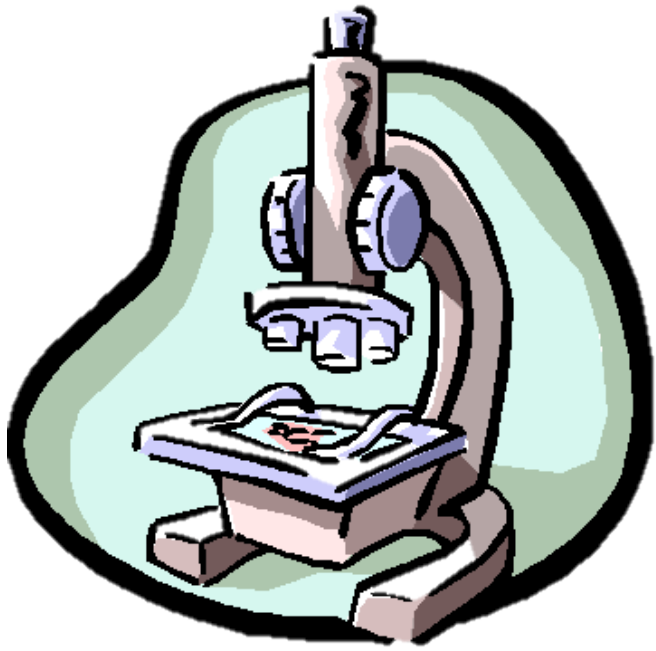
The replication cycle of poxvirus.
(Figure from McFadden G. Nat. Rev. Microbiol. 2005 Mar; 3(3): 201-13)



Materials and methods

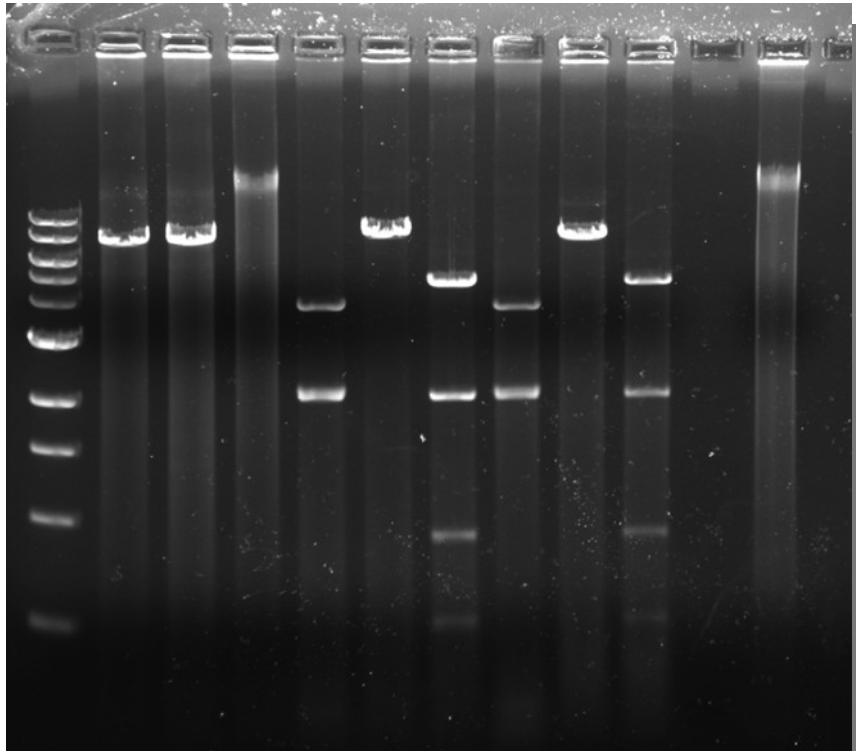
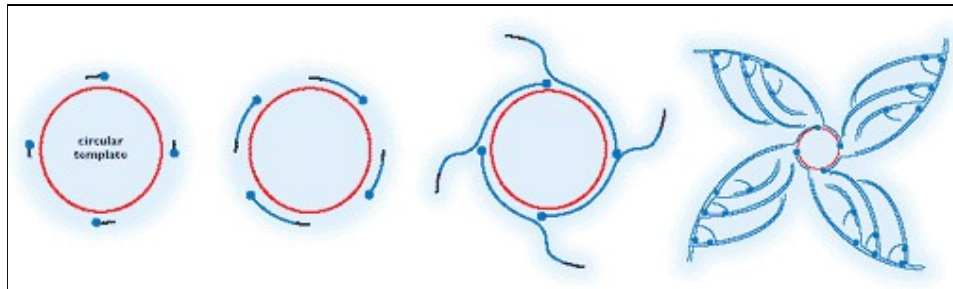
Samples collected between 2011 and 2014.





Rolling circle amplification (RCA)

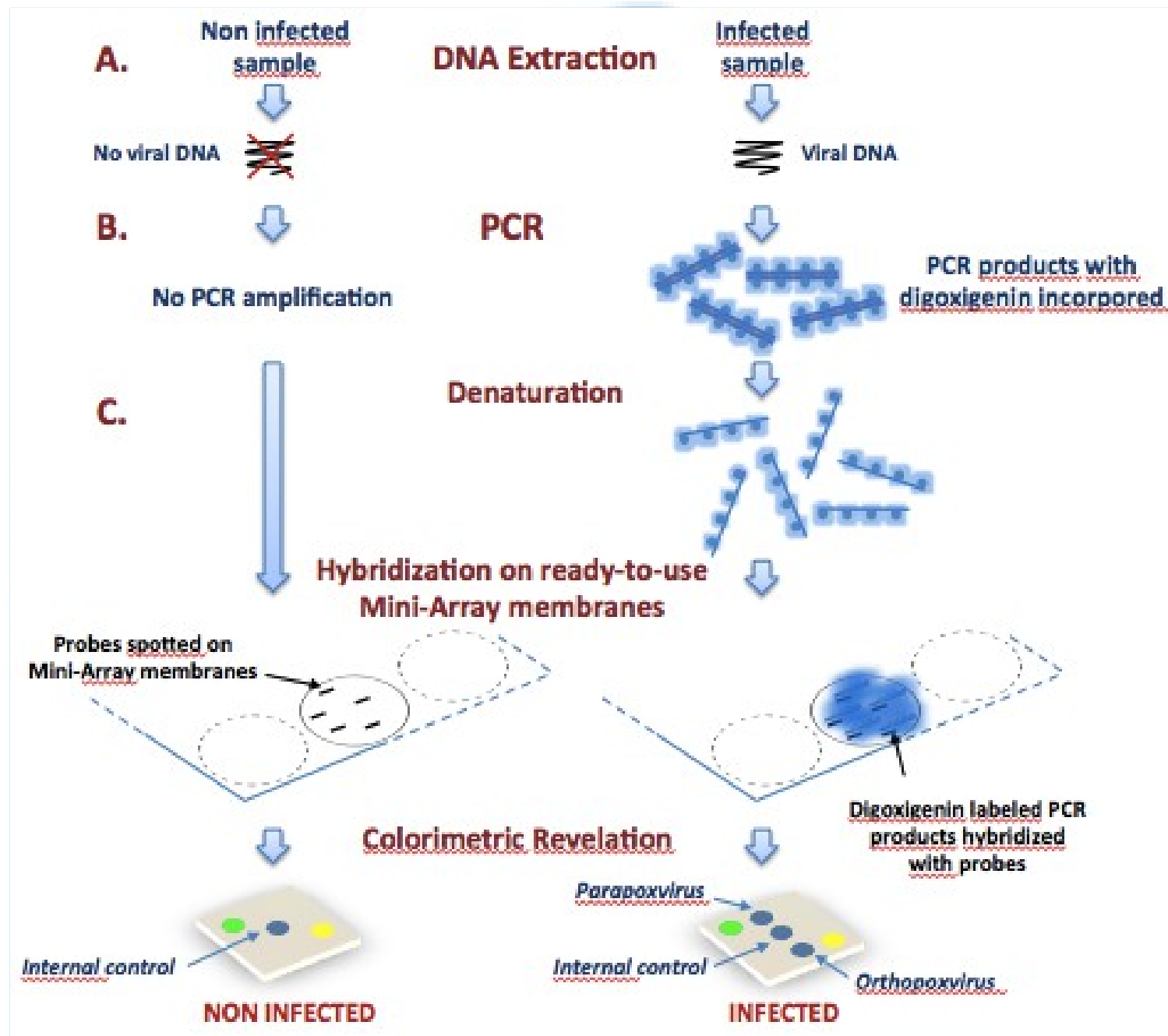
DNA polymerase phage Phi 29 B. subtilis

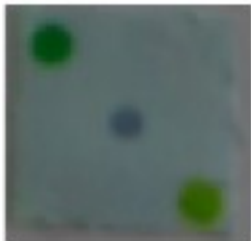
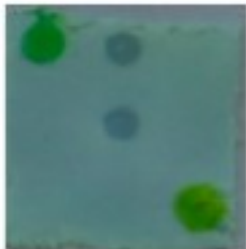
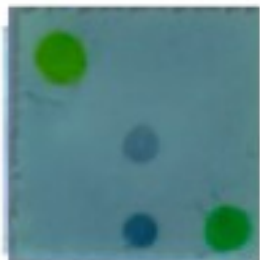



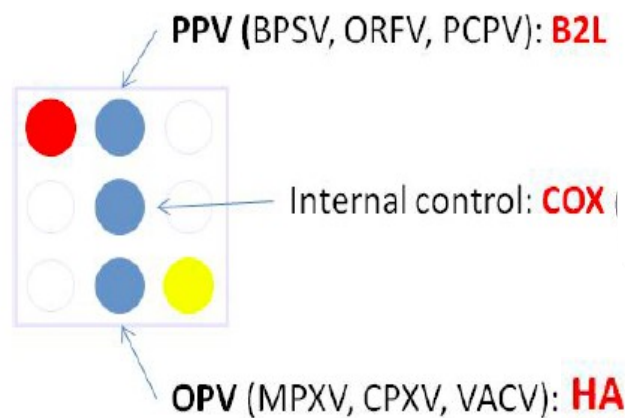
PCRs and Sequencing

- **FAP 59-64** (Forslund et al. 1999) to identify different papillomavirus in humans and in animals.
- **B1/2-E5 B1/2-E5** (Brandt et al. 2008) to identify *Delta-Papillomavirus* (BPV-1 e BPV-2).
- **BPV3-10F/R** (Brandt et al., 2011) to identify *Xi-Papillomavirus* (BPV-3, BPV-4, BPV-6, BPV-9 e BPV-10).
- **BPV5-8F/R** (Brandt et al., 2011) to identify *Epsilon-Papillomavirus* (BPV-5 e BPV-8).





COX positive PPV OPV Negative	PPV COX positive
	
OPV COX positive	PPV COX OPV positive
	

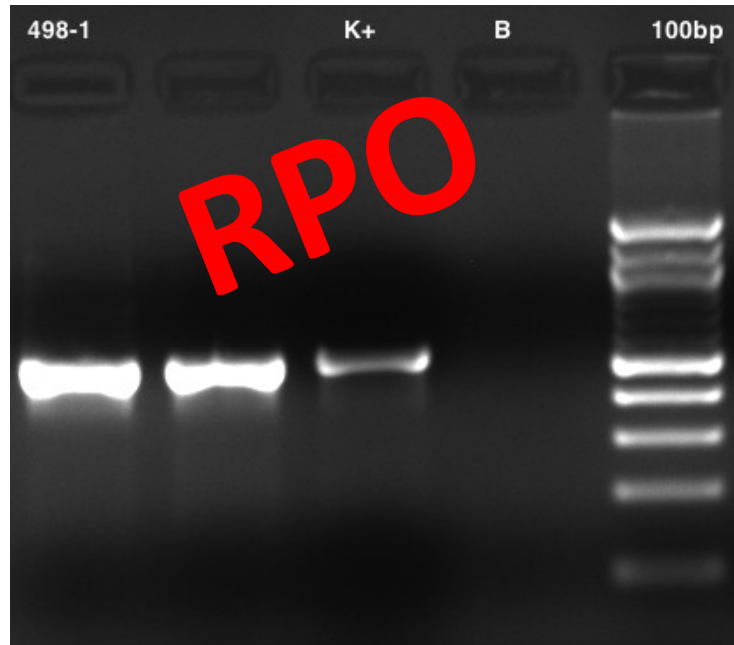
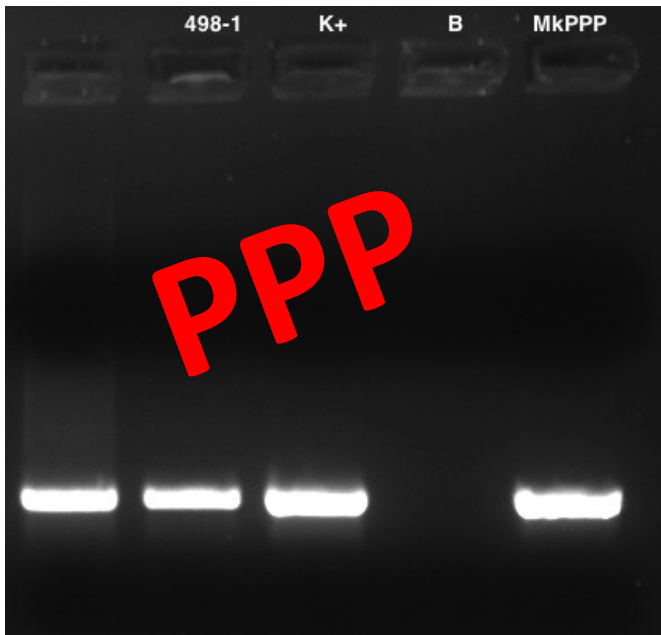
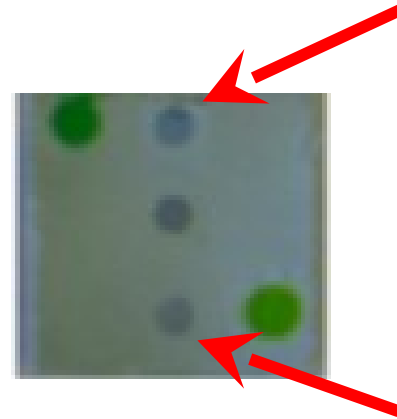


All the Mini array positive samples were amplified by:

PPPI and PPPIV (Hinoshima *et al.*, 2000) specific for *Parapoxvirus* B2L gene


RPOF1 and f2 (Ropp *et al.*, 1995) specific for the DNA dependent RNA polymerase of *Orthopoxvirus*

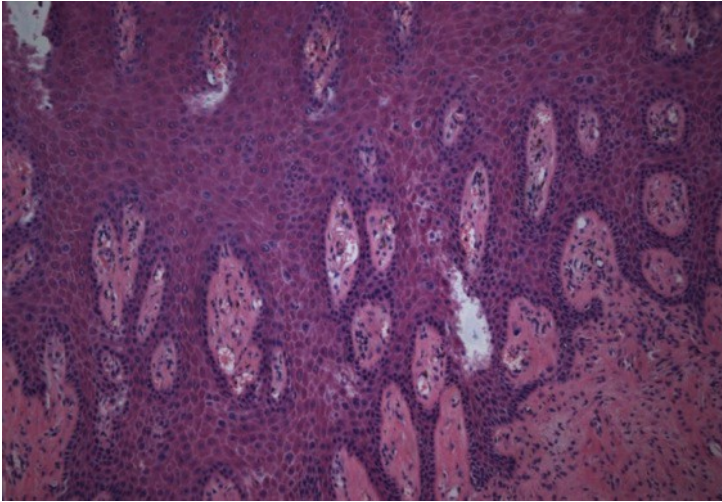
FOR SUBSEQUENT SEQUENCING



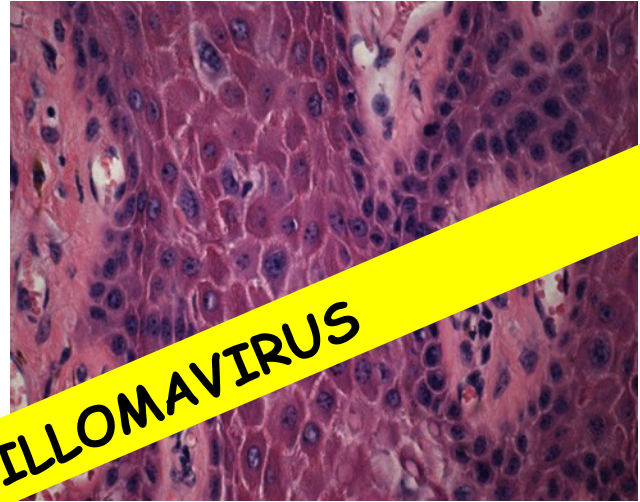


RESULTS

ID	δ-Papillomavirus	ξ -Papillomavirus	ε -Papillomavirus	Parapoxvirus	Orthopoxvirus
25084- 161	BPV-1	-	-	-	
31585- 156	BPV-1	BPV-6			
75176- 153	BPV-2				
85704- 162	BPV-2				
10549- 174		+			
28580- 287	BPV-1	+ 78 % identità con BPV-4		BPSV	
6231-416	BPV-1	+	BPV-8	PCPV	
14647-429	BPV-1	+ 74% identità nucleotidica con BPV-10		BPSV	+
33225-498	BPV-2	+		PCPV	+
50627-534	BPV-1	BPV-6		BPSV	
1818	-	BPV-6		PCPV	
12748- 610	BPV-1			PCPV	

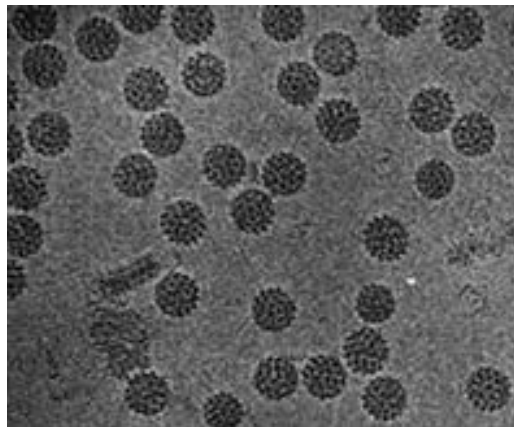


10 X



40 X

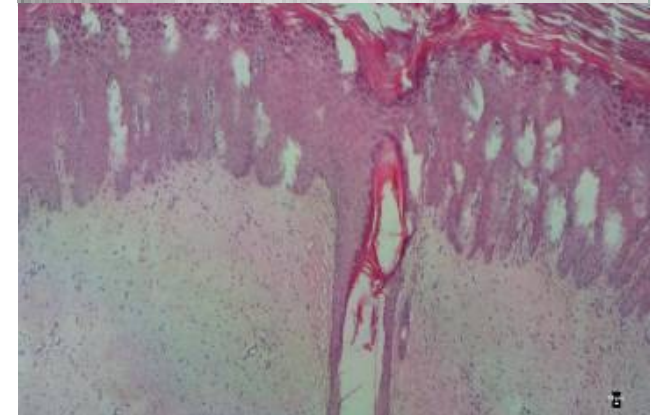
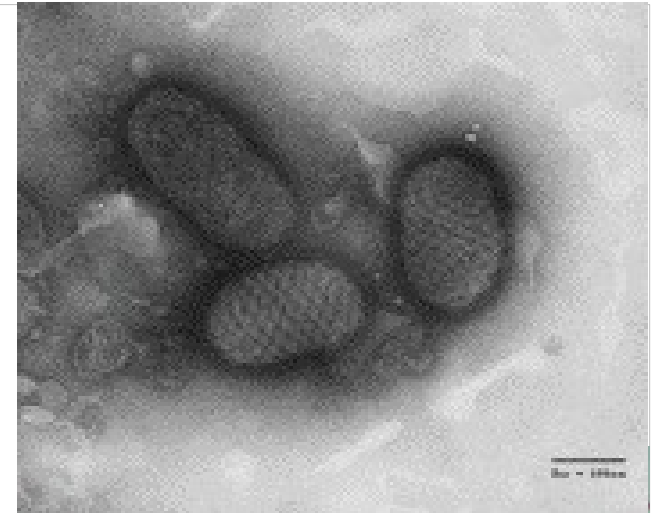
Principal etiologic agent Delta - PAPILLOMAVIRUS





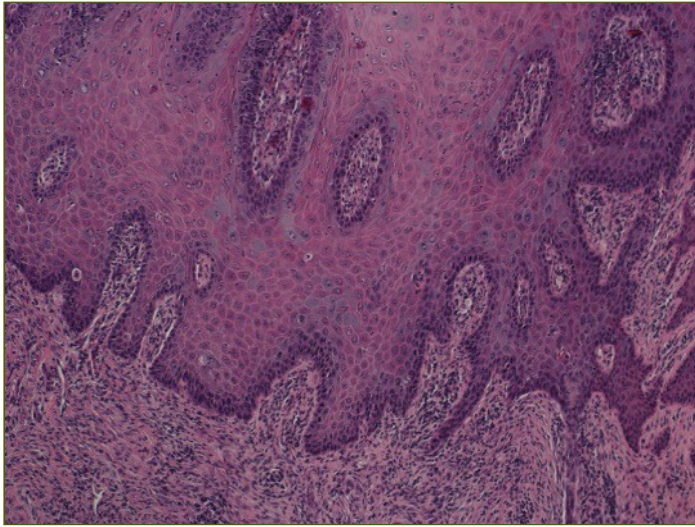
RESULTS

ID	Papillomavirus	Parapoxvirus	Orthopoxvirus
70452- 158		+	
70461- 157		+	
73824- 159		+	
73830- 160		+	
13171- 163		-	
13173- 164		+	
13175- 305		+	
13176- 306	+ (OaPV 3)	+	
154	-	+	
54836- 349		+	
79157- 387		+	
60530- 359		-	
9605-425		+	+
32063-501		+	+
60945-544		-	
68248-569 1/2		+	
80197-573		-	
21646-624		-	



**Principal etiologic agent
PARAPOXVIRUS (OV)**

Only one case (13176-306) was found positive for OaPV-3



• OaPV - 3



SCC - Sardegna - Alberti *et al.*, 2010

Conclusions 1

1. Proliferative lesions clinically defined as "Papillomatosis" can be the result of co-infections with different epitheliotropic viral species.
2. Cows with proliferative lesions are frequently infected with δ *Papillomavirus*, associated or not with ξ and ϵ .
3. Many animals were found infected with ZOONOTIC *Parapoxvirus*
4. Atypical malignant lesions in sheep and goats may be the result of co-infections with immune suppressive agents such as Pestivirus and Lentivirus (CAEV e MVV)

Majority ATGTCGAGCTTTGTTACCAATGGATACC TTCCGTTACATTGGAACCTCATGAGCTGACGTTAGACATAAAACCTAATATTAGGAATGCCGTATATAAGACGTATCTCCATAGAGAAATT

10 20 30 40 50 60 70 80 90 100 110 120

VACV.seq 120

CPXV.seq 120

429.seq 120

498.seq 120

425.seqC..... 120

501.seqC.A.....A.C..... 120

Majority AGTGGTAAAATGGCCAAGAAAATAGAAATTCGTGAAGACGTGGAATTACCTCTCGGCGAAATAGTTAATAATTCTGTAGTTATAAACGTTCCGTGTGTAATAACCTACGCGTATTATCAC

130 140 150 160 170 180 190 200 210 220 230 240

VACV.seq 240

CPXV.seq 240

429.seq 240

498.seq 240

425.seq 240

501.seq 240

Majority GTTGGGGATATA..... 250

VACV.seq 360

CPXV.seq 360

429.seq 360

498.seq 360

425.seq 360

501.seq 360

Majority AAGTACTGCTTT..... 370

VACV.seqC..T..... 480

CPXV.seq 480

429.seq 480

498.seq 480

425.seqA..... 480

501.seqT.....C.....A.....T..... 480

Percent Identity

	1	2	3	4	5	6	
1		99.6	99.6	99.6	99.2	97.3	1
2	0.4		100.0	100.0	99.6	97.3	2
3	0.4	0.0		100.0	99.4	97.2	3
4	0.4	0.0	0.0		99.4	97.2	4
5	0.8	0.4	0.4	0.4		97.7	5
6	2.7	2.7	2.7	2.7	2.3		6
	1	2	3	4	5	6	

VACV seq

CPXY seq

429.seq

498.seq

425.seq

501.seq

.....C..T.....

.....TCGCGAATATCGTCGAC

.....470 480

.....480

.....480

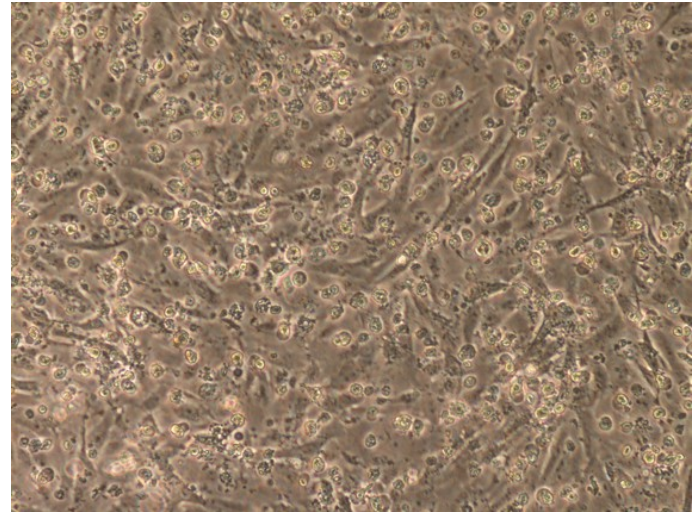
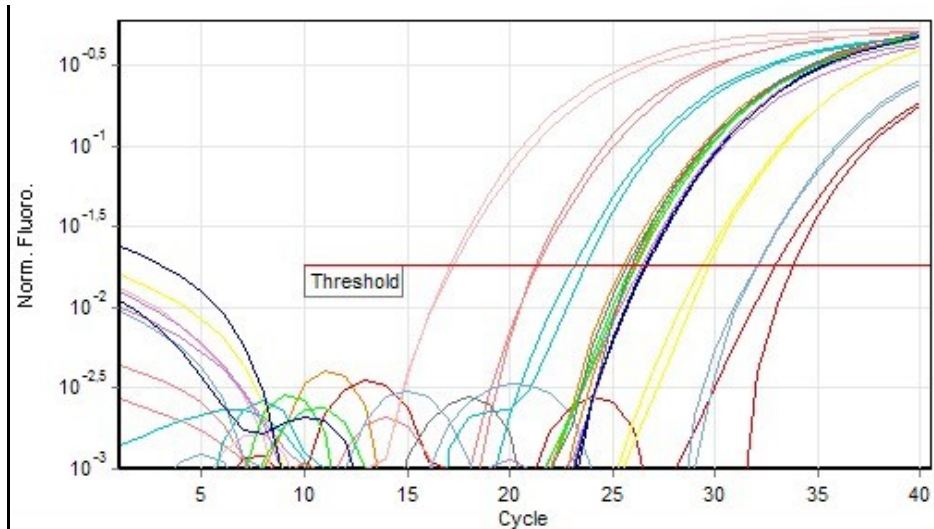
.....480

.....480

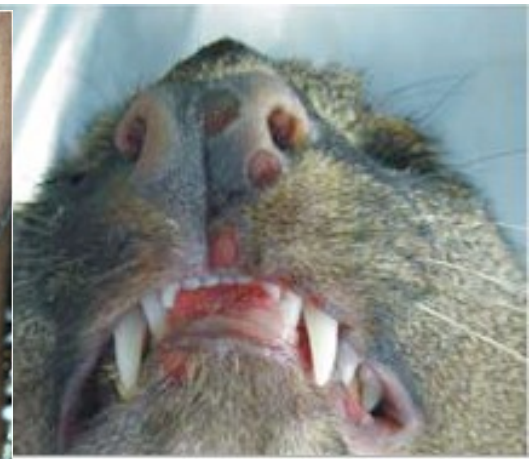
.....480

Conclusions 2

1. First report of CPXV infections in domestic ruminants in Italy
2. Co-infections with OPV and PPV either in cattle and in goats



VIRAL INTERFERENCE?



Acknowledgements



Istituto Zooprofilattico Sperimentale
della Lombardia e dell'Emilia Romagna

Thanks for your attention!



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