



"Endangered large carnivores and scavenging raptors in Europe"

Faculty of Veterinary Medicine, Teramo – 13-15 October 2016

FIRST DESCRIPTION OF AVIAN PAPILLOMAVIRUS INFECTION IN *GYPS FULVUS*, ITALY

Cristina E. Di Francesco, Francesca Profeta, Mariarita Romanucci,
Roberto Zuccarini, Tiziana Altea, Daniela Malatesta, Leonardo Della
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**FIRST DESCRIPTION OF AVIAN
PAPILLOMAVIRUS AND POXVIRUS
COINFECTION IN *GYPS FULVUS*,
ITALY**

Cristina E. Di Francesco, Francesca Profeta, Mariarita Romanucci,
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CASE REPORT

- September 2015
- “Riserva naturale del Monte Velino”
- Population of griffons (30-35 couples-140 animals)
- Affected /examined animals 9/61 (14.3%)
 - 6 adults and 3 subadults
 - 3 females and 6 males
- Multifocal, frequently ulcerated, cutaneous papulopustular and nodular lesions, with superficial crusts
- Flat, round cutaneous ulcers



MATERIALS AND METHODS- Histology

- 4 samples of cutaneous lesions collected from 2 animals
- Tissues were fixed in 10% neutral buffered formalin and embedded in paraffin wax
- Sections of 4 μm were stained with haematoxylin and eosin



MATERIALS AND METHODS– Electron Microscopy

- Formalin-fixed samples were prepared for electron microscopy
- After rehydration, tissues were re-fixed in glutaraldehyde solution (2.5%) and embedded in epoxy resin
- Ultra-thin sections were stained with uranyl acetate and lead citrate, and examined with a Zeiss EM 109 transmission electron microscope



MATERIALS AND METHODS - Nucleic acid isolation

- Cutaneous samples from 2 animals
- Stored at -20° C
- Tissues were homogenised (10%) in PBS pH 7.2
- DNA was extracted using the Dneasy Tissue Kit (Qiagen)
 - Proteinase K overnight at 56° C



MATERIALS AND METHODS - Nucleic acid isolation

- PCR protocols for Avipoxvirus and Avian Papillomavirus
- Avipoxvirus Gene *CNPV240* (Core protein P4b) – 250 and 578 bp

Name Primer	Sequence	Reference
Multiplex Poxvirus (F)	5'- GATGGCTGACGAGGAACAAAT -3'	Pérez-Trís <i>et al.</i> , 2011
Multiplex Poxvirus (R)	5'- TAGCCGGCATAAACATAACTCTTC-3'	
Poxvirus P4b (F)	5'-CAGCAGGTGCTAAACAACAA-3'	Lee & Lee, 1997
Poxvirus P4b (R)	5'- CGGTAGCTTAACGCCGAATA -3'	

- Gene *L1* major capsid protein of Avian papillomaviruses – 435 bp

Name Primer	Sequence	Reference
Multiplex Papillomavirus (F)	5'-TYCCWAAGGTSTCTGSAAATCA-3'	Pérez-Trís <i>et al.</i> , 2011
Multiplex Papillomavirus (R)	5'- -CCRAAGCCAATATCKSACAT-3'	

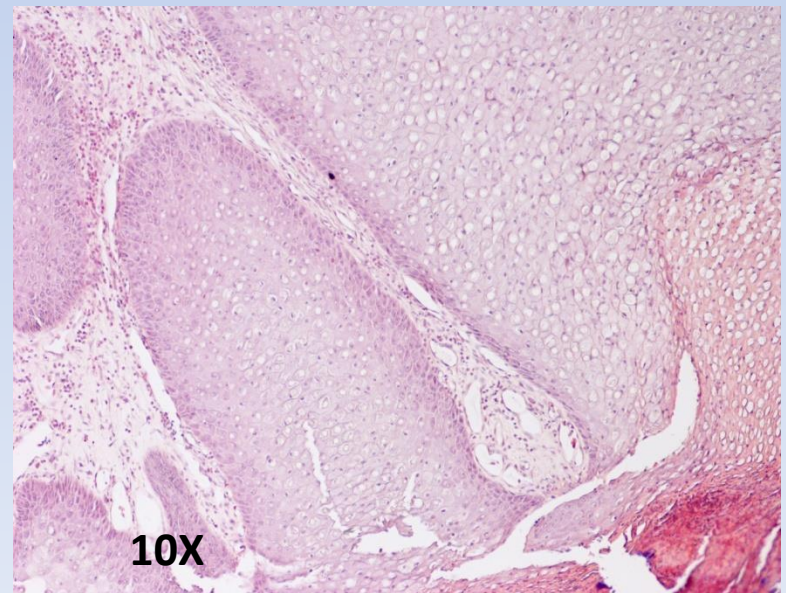
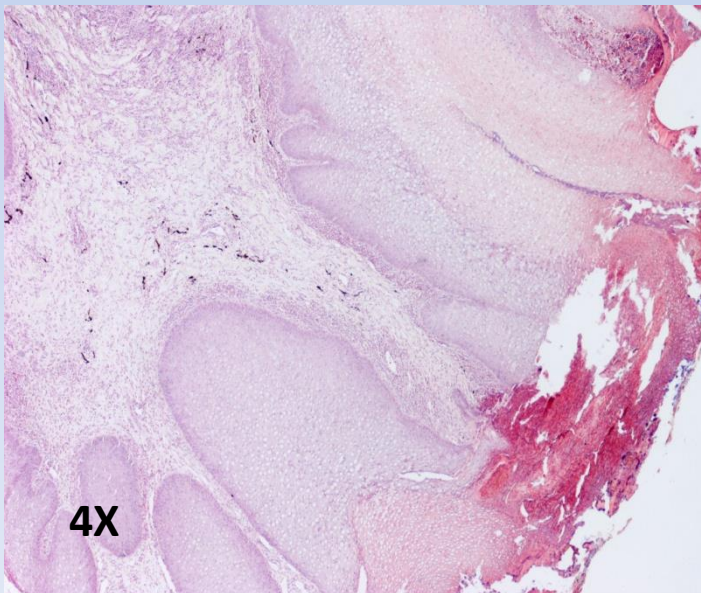
MATERIALS AND METHODS– Sequence analysis

- Fragments were purified by PCR Extraction kit (Qiagen)
- DNA sequencing at BioFab research srl laboratories
 - For Papillomavirus, additional degenerated primers were designed on the basis of the sequences of avian papillomaviruses
- Sequences were compared in GenBank database using Clustal Omega and Blast softwares
- Phylogenic analysis was performed by MEGA.5 software



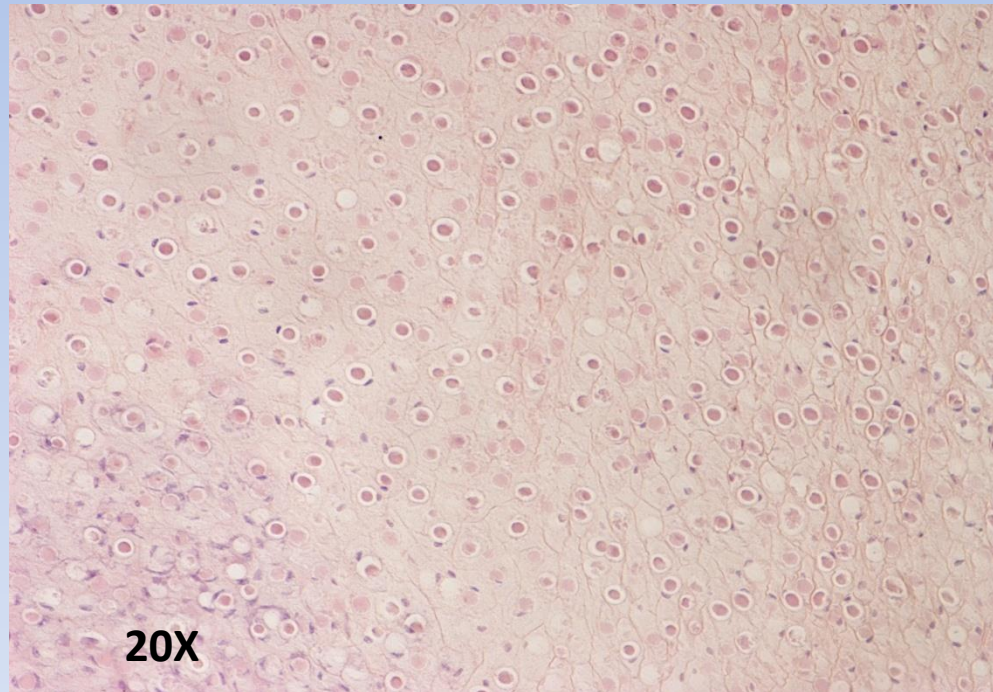
RESULTS- Histology

- Intense and diffuse epidermal hyperplasia (4X)
- Multifocal to diffuse areas of epidermal ulceration (4X)
- Intense heterophilic infiltration and diffuse serocellular crusts with mixed bacterial colonies (4X)
- Multifocal, perivascular lymphocytic infiltration in the dermis (4X)
- Keratinocytes with extensive vacuolization and swelling (10X)



RESULTS- Histology

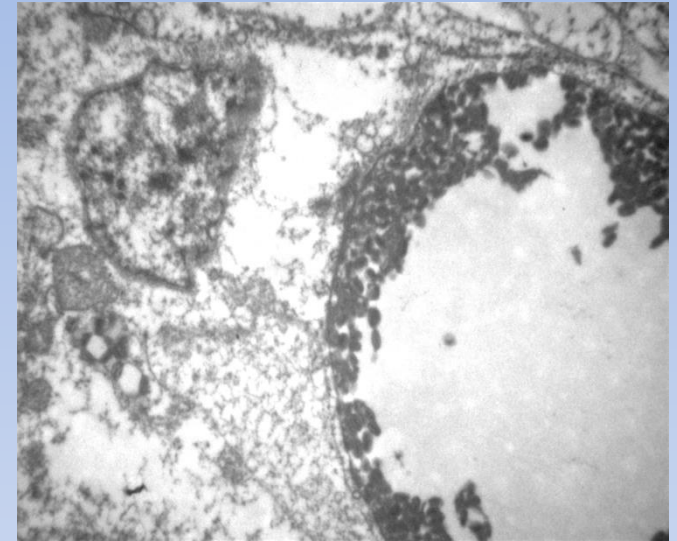
- Numerous eosinophilic cytoplasmic inclusion bodies characteristic of Poxvirus (Bollinger bodies)



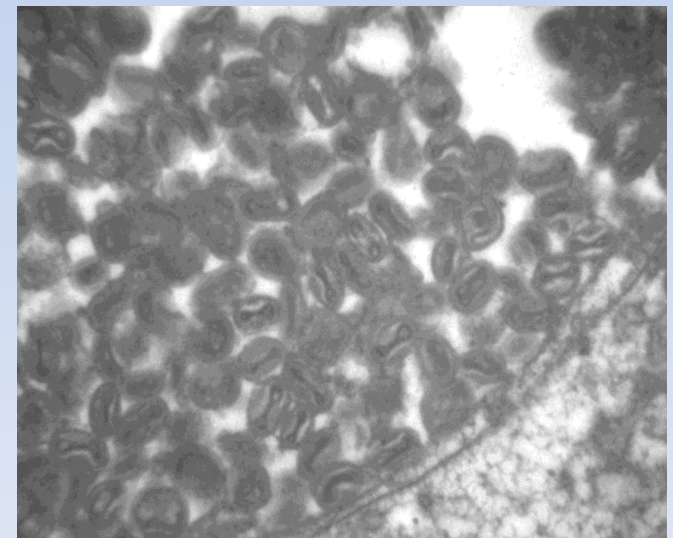
- No other findings, nuclear inclusions, or koilocytes typical of papillomavirus infection

RESULTS – Electron Microscopy

- Evident viral inclusion within the cytoplasm of an epidermal cell containing several virions (**3.000x**)



- Detail of inclusion with numerous mature virions of 250-300 nm
- Enveloped virions showing electron dense core with a characteristic dumbbell-shaped structure (**20.000x**)



RESULTS– Nucleic acid isolation

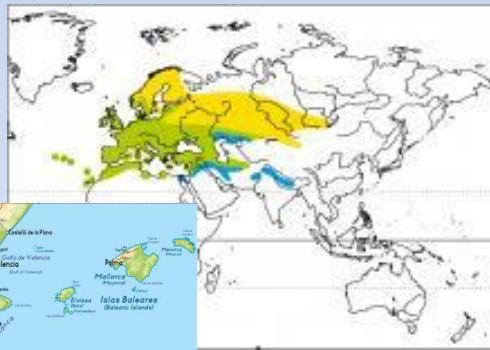
- A fragment of 243 bp was obtained with primers **MultiplexPoxvirus**
- No amplification with primers Poxvirus P4b
- A final fragment of 528 bp was obtained with **MultiplexPapillomavirus** and degenerated primers

RESULTS– Sequence analysis

- **Poxvirus primers:**
 - Sequence specific for gene *P4b* of Avipoxvirus
 - Highest identity with Penguinpox virus (92%)
 - Similar identity (92%) but with a lower coverage (80%) for Avipox isolate ID8964



Chaffinch (*Fringilla coelebs*)



African penguin (*Spheniscus demersus*)



RESULTS– Sequence analysis

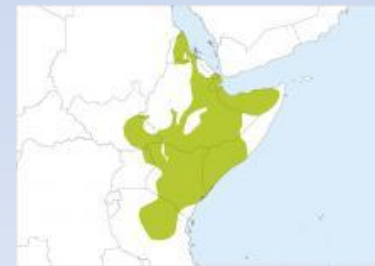
- **Papillomavirus primers**

- Sequence specific for *L1* gene of avian Papillomaviruses

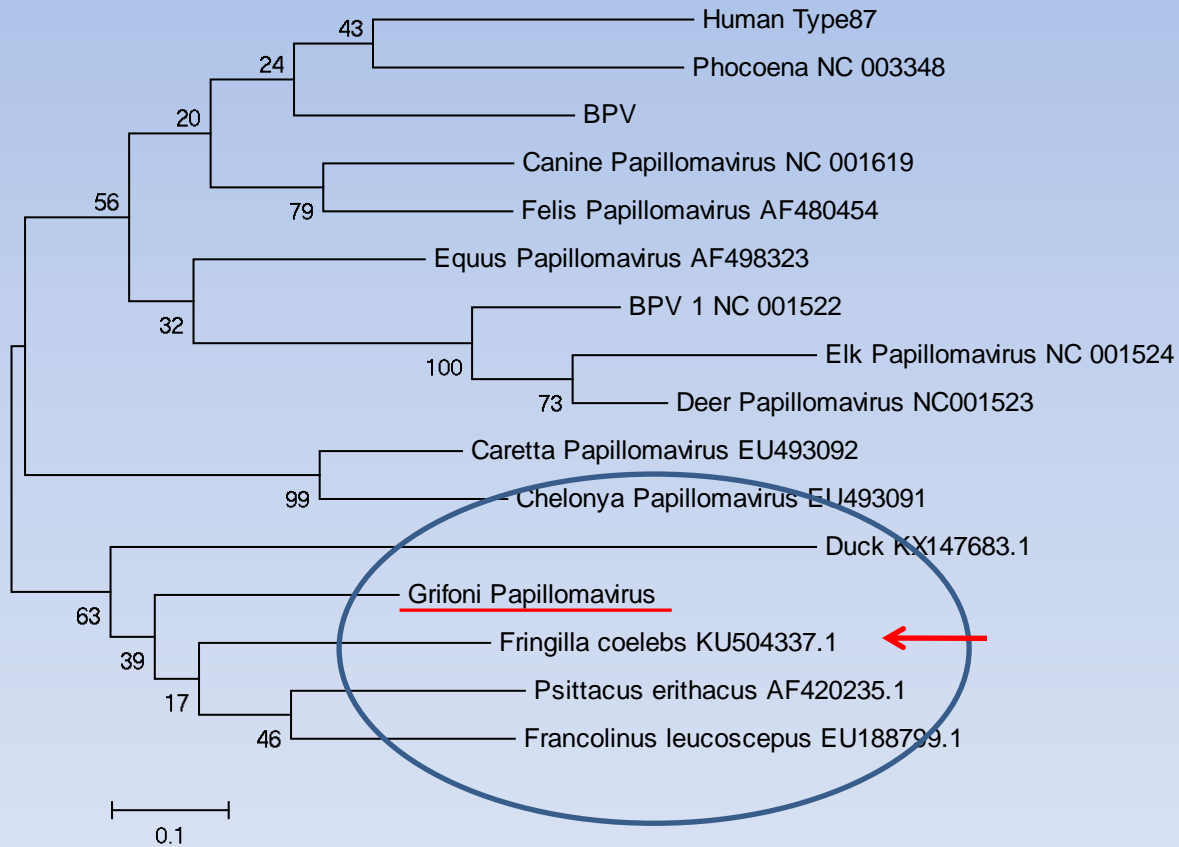
- Highest identity with *Psittacus erithacus* papillomavirus PePV (69%; coverage 99%) and Duck papillomavirus (68%; coverage 99%)



- Lower identity for *Francolinus leucoscepus* papillomavirus 1 FIPV1 (66%; coverage 94%)



RESULTS – Phylogenetic analysis



DISCUSSION

- First identification of Avipoxvirus and Avian papillomavirus in griffon vulture
- Avipoxvirus
- Avian papillomavirus



Table 1 (continued) A.Rector, M.VanRanst/Virology 445 (2013) 213–

Host species taxonomic order	Host species	223	Papillomavirus name	Abbreviation (previous)	Classification
	Rickett's big-footed bat	<i>Myotis ricketti</i>	<i>Myotis ricketti</i> papillomavirus 1	MrPV1	unclassified
	Egyptian fruit bat	<i>Rousettus aegyptiacus</i>	<i>Rousettus aegyptiacus</i> papillomavirus 1	RaPV1	Psi-papillomavirus 1
Diprotodontia	Brush-tailed bettong	<i>Bettongia penicillata</i>	<i>Bettongia penicillata</i> papillomavirus 1	BpPV1	Dyokappapapillomavirus 1
	European hedgehog	<i>Erinaceus europaeus</i>	<i>Erinaceus europaeus</i> papillomavirus 1	EePV1 (EHPV)	Dyoeatapapillomavirus 1
	Yellow-necked Frankolin	<i>Francolinus leucocepus</i>	<i>Francolinus leucocepus</i> papillomavirus 1	FIPV1 (FLPV)	Dyoeptilonpapillomavirus 1

DOI: 10.7559/2012-09-230 Journal of Wildlife Diseases, 49(4), 2013, pp. 978–985 © Wildlife Disease Association 2013

CHARACTERIZATION OF AVIAN POXVIRUS IN ANNA'S HUMMINGBIRD (*CALYPTE ANNA*) IN CALIFORNIA, USA

Loreto A. Godoy^{1†}, Lisa S. Dalbeck¹, Lisa A. Tell², Leslie W. Woods³, Rita R. Colwell⁴, Barbara Robinson⁴, Susan M. Wethington⁴, Anneke Moresco⁵, Peter R. Woolcock⁶, Holly B. Ernest^{1,6,7}



LETTERS

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and Characterization of *Fringilla coelebs* Papillomavirus in Free-living and Captive Birds in Italy

Mari, ^{1,3} Mariagrazia Zanoni¹, Laura Gallina², Giovanni Casa², Alessandra Mazza¹ ¹Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, 25124 Brescia, Italy; ²Dipartimento di Scienze Mediche Veterinarie, Alma Mater University, Via Tolara di sopra 50, 40064 Ozzano Emilia (Bologna), Italy; ³Corresponding author

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AVIAN POXVIRUS INFECTION IN A FLAMINGO (*PHOENICOPTERUS RUBER*) OF THE LISBON ZOO

Ana M. Henriques, Ph.D., Teresa Fagulha, D.V.M., Margarida Duarte, D.V.M., Mariana Ramos, D.V.M., Sílvia C. Barros, Ph.D., Tiago Luís, B.Sc., Rui Bernardino, D.V.M., Mariana Fernandes, D.V.M., Narciso Lapão, D.V.M., José Ferreira da Silva, D.V.M., Ph.D., and António Faveiro, D.V.M., Ph.D.



Claudia Niemeyer¹, Cíntia M. Favero², Cristiane K. M. Kolesnikovas³, Renata Paulo Brandão² and José Luiz Catão-Dias¹

Duerr³, F. N. Dela Cruz Jr.¹,



DISCUSSION

- First report of co-infection in wild birds

Avian Pathology, 2014

Vol. 43, No. 2, 130–134, <http://dx.doi.org/10.1080/03079457.2014.886326>

ORIGINAL ARTICLE

Polymerase chain reaction detection of avian papillomavirus in naturally infected blood, swab and tissue samples

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Table 1. Summary of PCR tests for cytochrome b, avipox/avian papillomavirus multiplex and simple avipox.

Species	Blood			Swab			Tissue		
	Cyt b	Multiplex (APV/PV)	Simple (APV)	Cyt b	Multiplex (APV/PV)	Simple (APV)	Cyt b	Multiplex (APV/PV)	Simple (APV)
<i>Carduelis carduelis</i>	(+)	(-)/(-)		(+)	(-)/(-)				
<i>Carduelis carduelis</i>	(+)	(-)/(-)		(+)	(-)/(-)				
<i>Cyanistes caeruleus</i>	(+)	(-)/(-)					(+)	(+)(-)	(+)
<i>Erithracus rubecula</i>	(+)	(-)/(-)		(+)	(+)(-)	(+)			
<i>Fringilla coelebs 1</i>	(+)	(-)/(-)					(+)	(-)/(+)	(-)
<i>Fringilla coelebs 2</i>	(+)	(-)/(-)					(+)	(-)/(+)	(-)
<i>Fringilla coelebs 3</i>	(+)	(-)/(-)					(+)	(-)/(+)	(-)
<i>Fringilla coelebs 4</i>	(+)	(-)/(-)					(+)	(-)/(+)	(-)
<i>Garrulus glandarius</i>	(+)	(-)/(-)		(+)	(-)/(-)				
<i>Parus major</i>	(+)	(-)/(-)		(+)	(-)/(-)				
<i>Parus major</i>	(+)	(-)/(-)		(+)	(-)/(-)				
<i>Passer domesticus 1</i>	(+)	(-)/(-)		(+)	(-)/(-)	(-)	(+)	(+)(-)	(+)
<i>Passer domesticus 2</i>	(+)	(-)/(-)		(+)	(-)/(-)				
<i>Passer domesticus 3</i>	(+)	(-)/(-)		(+)	(-)/(-)	(-)	(+)	(+)(-)	(+)
<i>Passer domesticus 4</i>	(+)	(-)/(-)		(+)	(+)(-)	(+)			
<i>Prunella modularis</i>	(+)	(-)/(-)		(+)	(-)/(-)				
<i>Sitta europea</i>	(+)	(-)/(-)		(+)	(-)/(-)				
<i>Sitta europea</i>	(+)	(-)/(-)		(+)	(-)/(-)				
<i>Sylvia atricapilla 1</i>	(+)	(-)/(-)	(-)	(+)	(+)(-)	(+)	(+)	(+)(-)	(+)
<i>Sylvia atricapilla 2</i>	(+)	(-)/(-)	(-)	(+)	(+)(-)	(+)	(+)	(+)(-)	(+)
<i>Sylvia atricapilla 3</i>	(+)	(-)/(-)	(-)	(+)	(+)(-)	(+)	(+)	(+)(-)	(+)
<i>Sylvia atricapilla 4</i>	(+)	(-)/(-)					(+)	(-)/(-)	(-)
<i>Sylvia atricapilla 5</i>	(+)	(-)/(-)					(+)	(+)(-)	(+)
<i>Sylvia atricapilla 6</i>	(+)	(-)/(-)		(+)	(-)/(-)				
<i>Sylvia atricapilla 7</i>	(+)	(-)/(-)		(+)	(+)(-)	(-)			
<i>Sylvia atricapilla 8</i>	(+)	(-)/(-)		(+)	(-)/(-)				
<i>Sylvia atricapilla 9</i>	(+)	(-)/(-)		(+)	(+)(-)	(-)			
<i>Sylvia atricapilla 10</i>	(+)	(-)/(-)		(+)	(-)/(-)				
<i>Sylvia atricapilla 11</i>				(+)	(+)(-)	(+)	(+)	(+)(-)	(+)
<i>Sylvia atricapilla 12</i>	(+)	(-)/(-)					(+)	(+)(-)	(+)
Total tested		29			22			14	
Cyt b-positive		29 (100%)			22 (100%)			14 (100%)	
APV-positive		0 (0%)			8 multiplex PCR (36%)			9 multiplex PCR (64%)	
					6 simple PCR (27%)			9 simple PCR (64%)	
PV-positive		0 (0%)			0 (0%)			1 (29%)	

APV, avipox; Cyt b, cytochrome b; PV, avian papillomavirus.

FUTURE PROSPECTIVES

- Pathogenic role of these infections in griffon???
- No mortality episodes
- Progressive and complete recover of affected animals



FUTURE PROSPECTIVES

- Pathogenic role of these infections in griffon???
- Monitoring of morbidity and mortality rate of associated Poxvirus/Papillomavirus is essential
- More in depth characterization of two viruses to understand the taxonomy (novel species? novel host?)

ACKNOWLEDGEMENTS

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- Mario Posillico



THANK YOU FOR THE ATTENTION