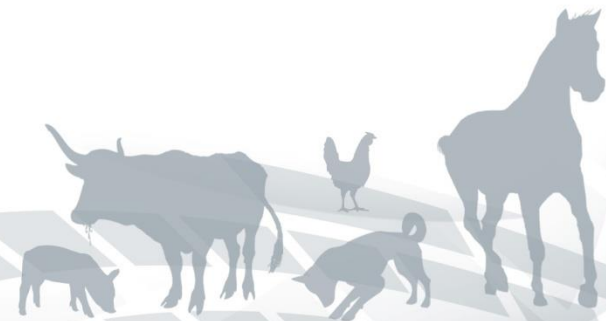




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Canine Distemper

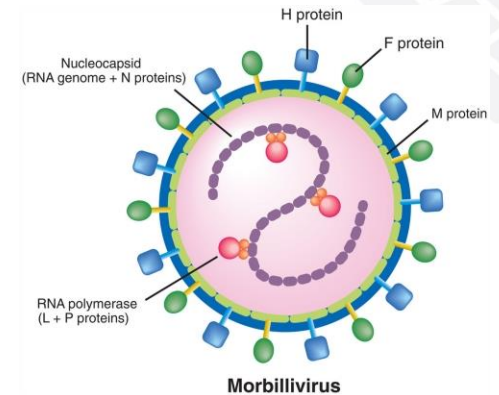


Famiglia Paramixoviridae

Genere Morbillivirus

Carrè, 1905

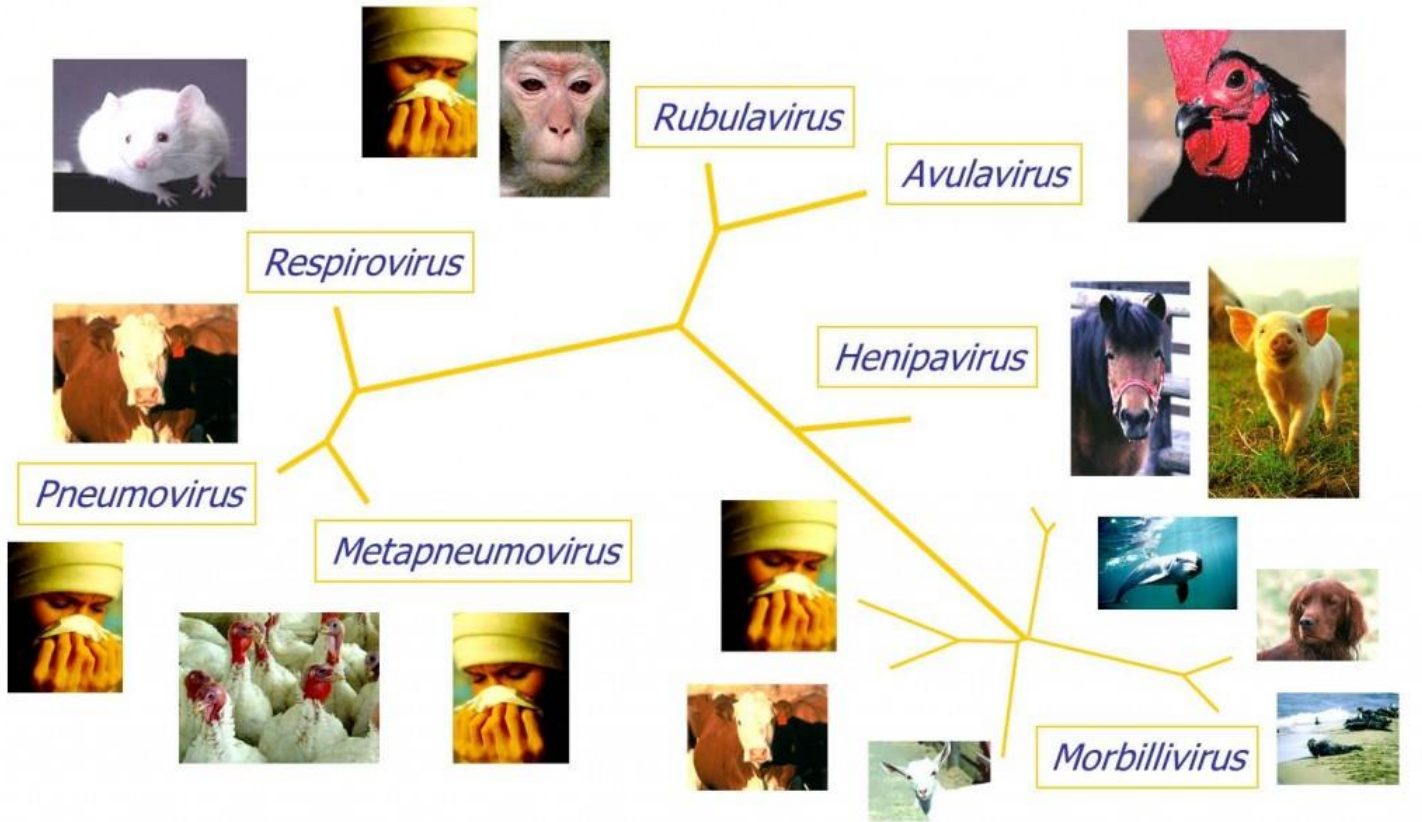
Decorso Acuto e Subacuto



MV eradication?

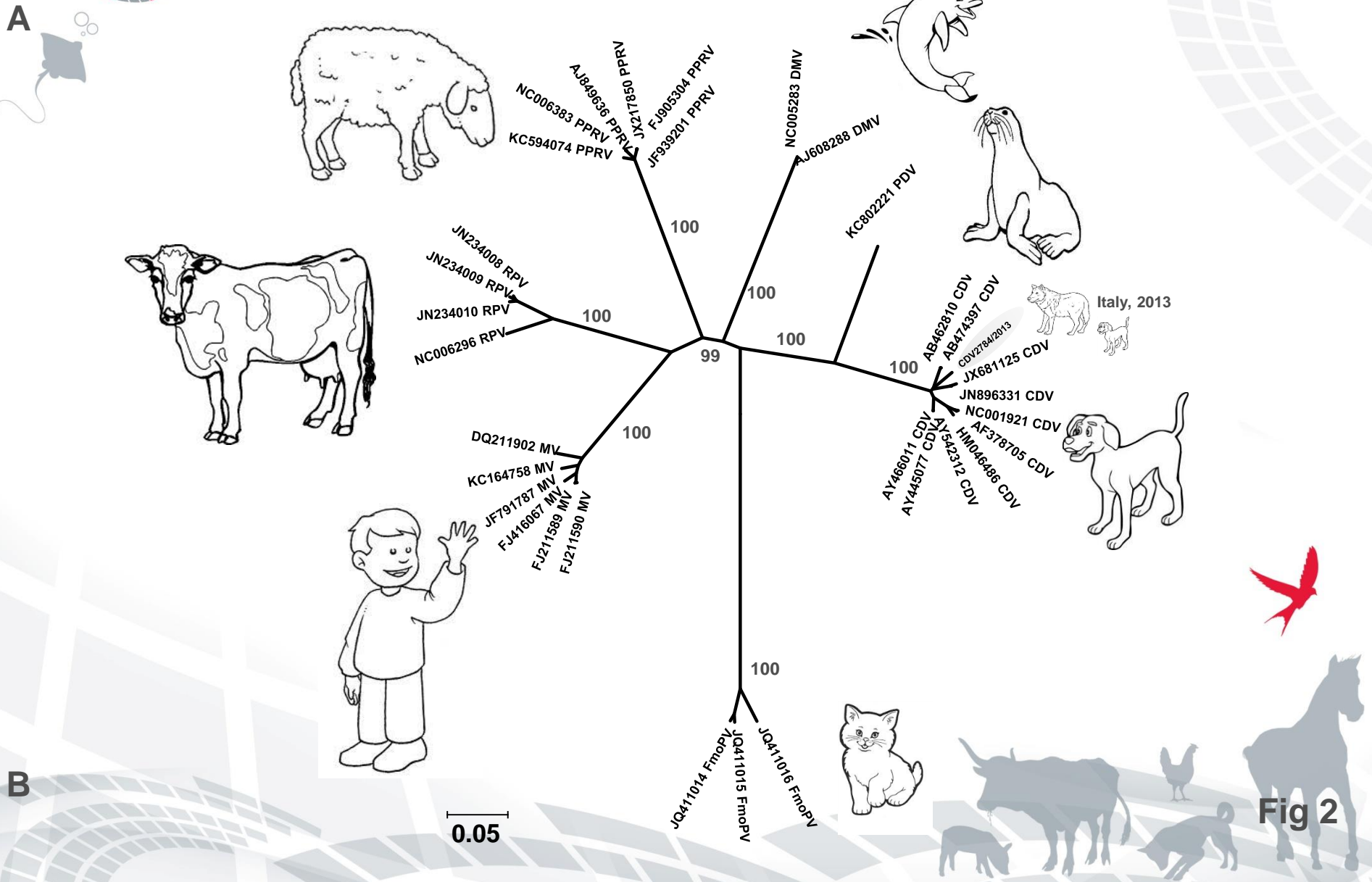
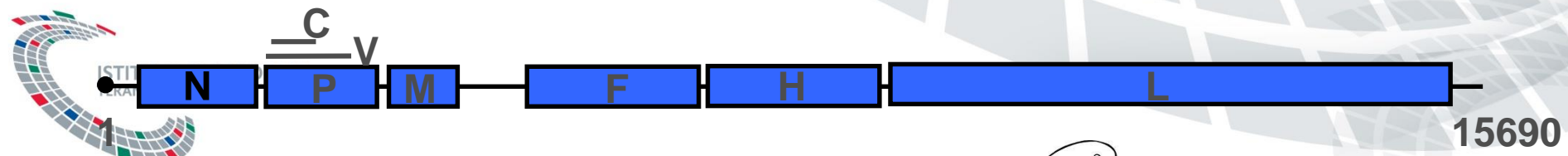
RV, MV, PPRV, DMV, PMV





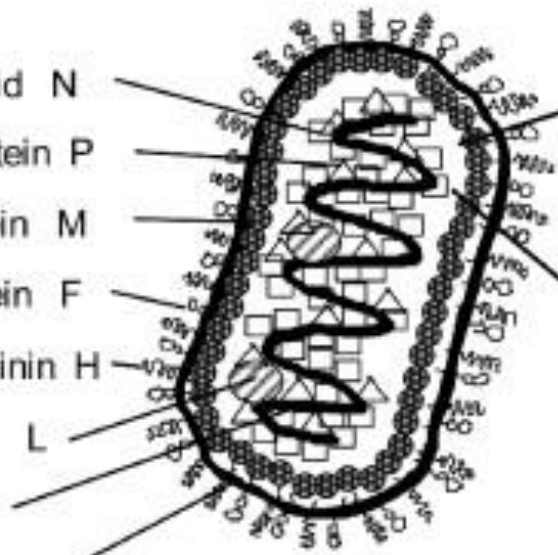
Paramyxoviridae



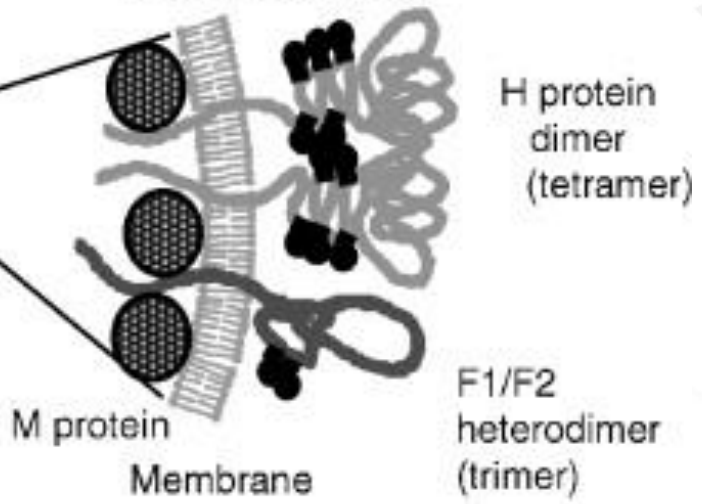


Virus particle

- Nucleocapsid N
- △ Phosphoprotein P
- Matrix protein M
- ↯ Fusion protein F
- ↯ Haemagglutinin H
- ⊙ Polymerase L
- ~ RNA
- Membrane



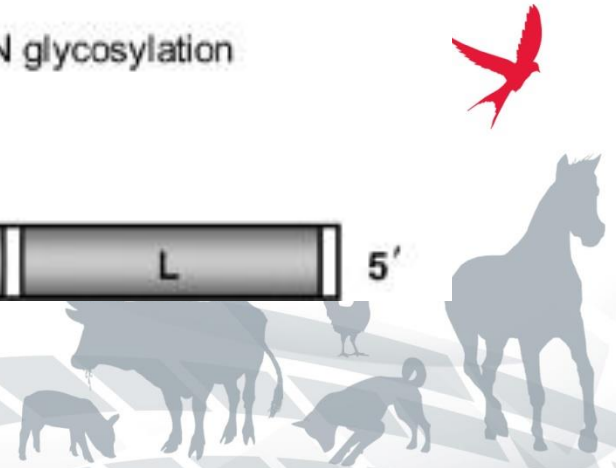
Envelope proteins



● = N glycosylation



Genome organisation: ss (-) RNA



VIRUS LABILE A DIFFERENZA DI CPV-2



Cani tutte età
Carnivori selvatici, mustelidi
Spill-over in altri animali



Canine Distemper Virus: an Emerging Disease in Wild Endangered Amur Tigers (*Panthera tigris altaica*)

JOURNAL
OF
THE ROYAL
SOCIETY

Interface

J. R. Soc. Interface (2007) 4, 1127–1134
doi:10.1098/rsif.2007.0235
Published online 24 April 2007

The canine distemper epidemic in Serengeti: are lions victims of a new highly virulent canine distemper virus strain, or is pathogen circulation stochasticity to blame?

Micheline Guiserix*, Narges Bahi-Jaber, David Fouchet, Frank Sauvage

DOI: 10.7559/2013-02-027

Journal of Wildlife Diseases, 49(4), 2013, pp. 1024–1027
© Wildlife Disease Association 2013

First Report of Clinical Disease Associated with Canine Distemper Virus Infection in a Wild Black Bear (*Ursus americana*)

Article | OPEN

Fatal canine distemper virus infection of giant pandas in China

Na Feng, Yicong Yu, Tiecheng Wang, Peter Wilker, Jianzhong Wang, Yuanguo Li, Zhe Sun, Yuwei Gao & Xianzhu Xia

Scientific Reports 6, Article number: 27518

(2016)

doi:10.1038/srep27518

Received: 27 October 2015

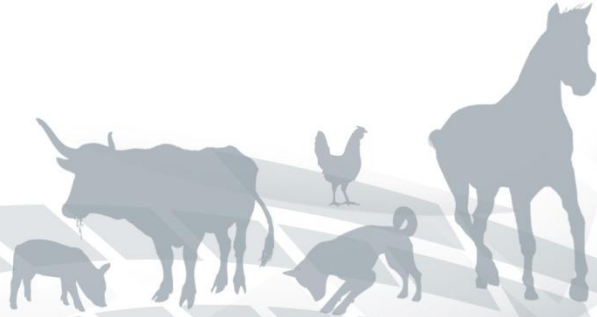
Accepted: 10 May 2016

Published online: 16 June 2016





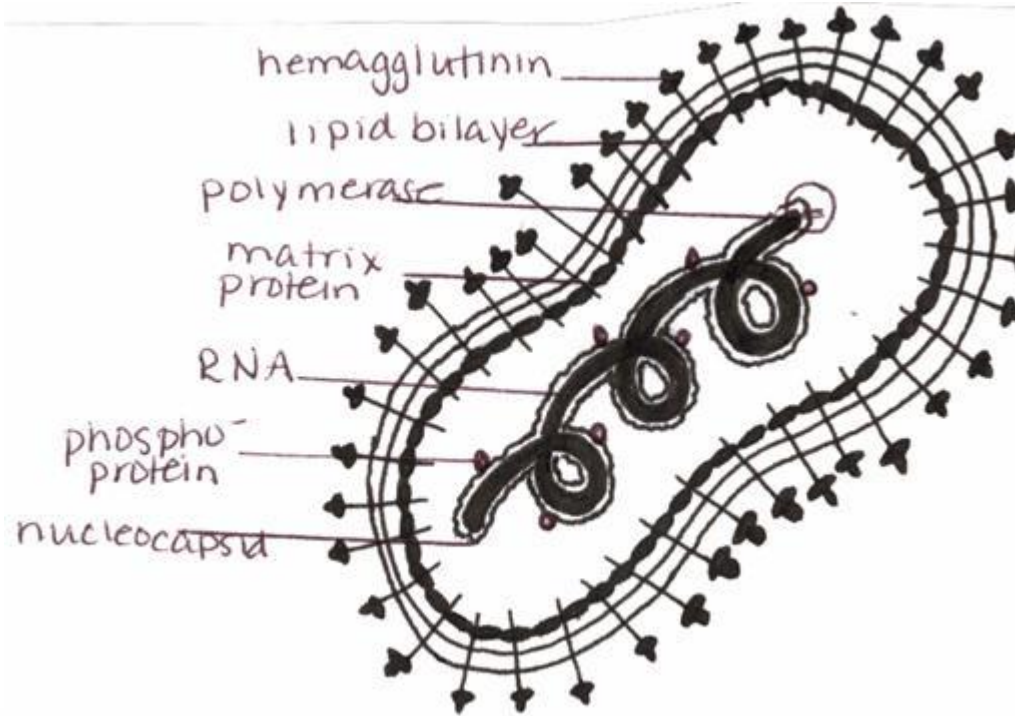
ISTITUTO G. CAPORALE
TERAMO

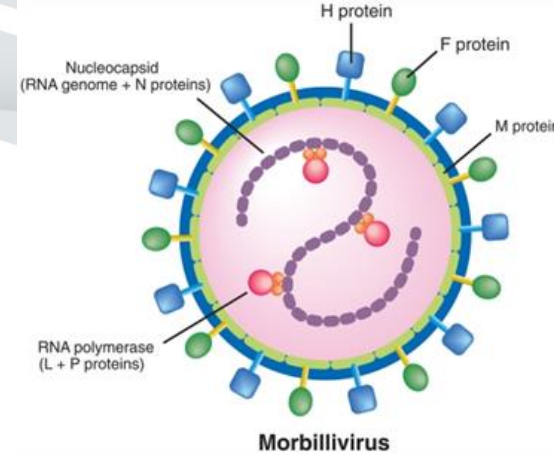




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Morbillivirus





RNA ss- Envelope

Simmetria Elicoidale

F, fusione membrana cellula ospite

H, adsorbimento

M, proteina interna

N, nucleoproteina

L, polimerasi

P, fosfoproteina



Disease	Geography of emergence	Impact on wildlife population	Refs
Plague	Panglobal, notably India, SW USA	Highly mortality in prairie dog "towns" during epizootics leading to declines in endangered black-footed ferret	<i>J. F. Cully, A. M. Barnes, T. J. Quan, G. Maupin, J. Wildl. Dis. 33, 706 (1997).</i>
Canine distemper	USA, Africa	Extinction of African wild dog and black-footed ferret; threat to Ethiopian wolf	<i>T. Thorne and E. S. Williams, Conserv. Biol. 2, 66 (1988). J. R. Ginsberg, G. M. Mace, S. Albon, Proc. R. Soc. London Ser. B. 262, 221 (1995); M. E. Roelke-Parker et al., Nature 379, 441 (1996); K. Laurenson, Anim. Conserv. 1, 273 (1998).</i>
Canine parvovirus disease	Europe, USA	Suspected cause of grey wolf population declines; threat to Ethiopian wolf	<i>I. McCandlish, H. Thompson, C. Cornwell, E. Fisher, Vet. Rec. 105, 180 (1979); C. R. Parrish et al., Science 230, 1046 (1985).</i>
Sarcopting mange	Australia, UK, Sweden	Recent threat wildlife in Sweden; emerging threat to wombats in Australia	<i>E. Lindström et al., Ecology 75, 1042 (1994); L. F. Skerratt, R. Martin, K. Handasyde, Austral. Vet. J. 76, 408 (1998).</i>

VIE DI TRASMISSIONE DEL VIRUS DEL CIMURRO

- **Aerosol**
- **Organi linfoidi (recettore)**
- **Epiteli (recettore) e CNS (8-12 g pi)**
- **Sintomi respiratori, intestinali, cutanei**
- **Sintomatologia nervosa (20 g pi)**
- **Immunosoppressione**

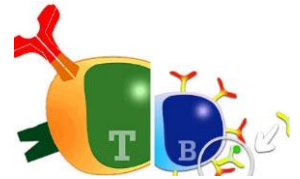




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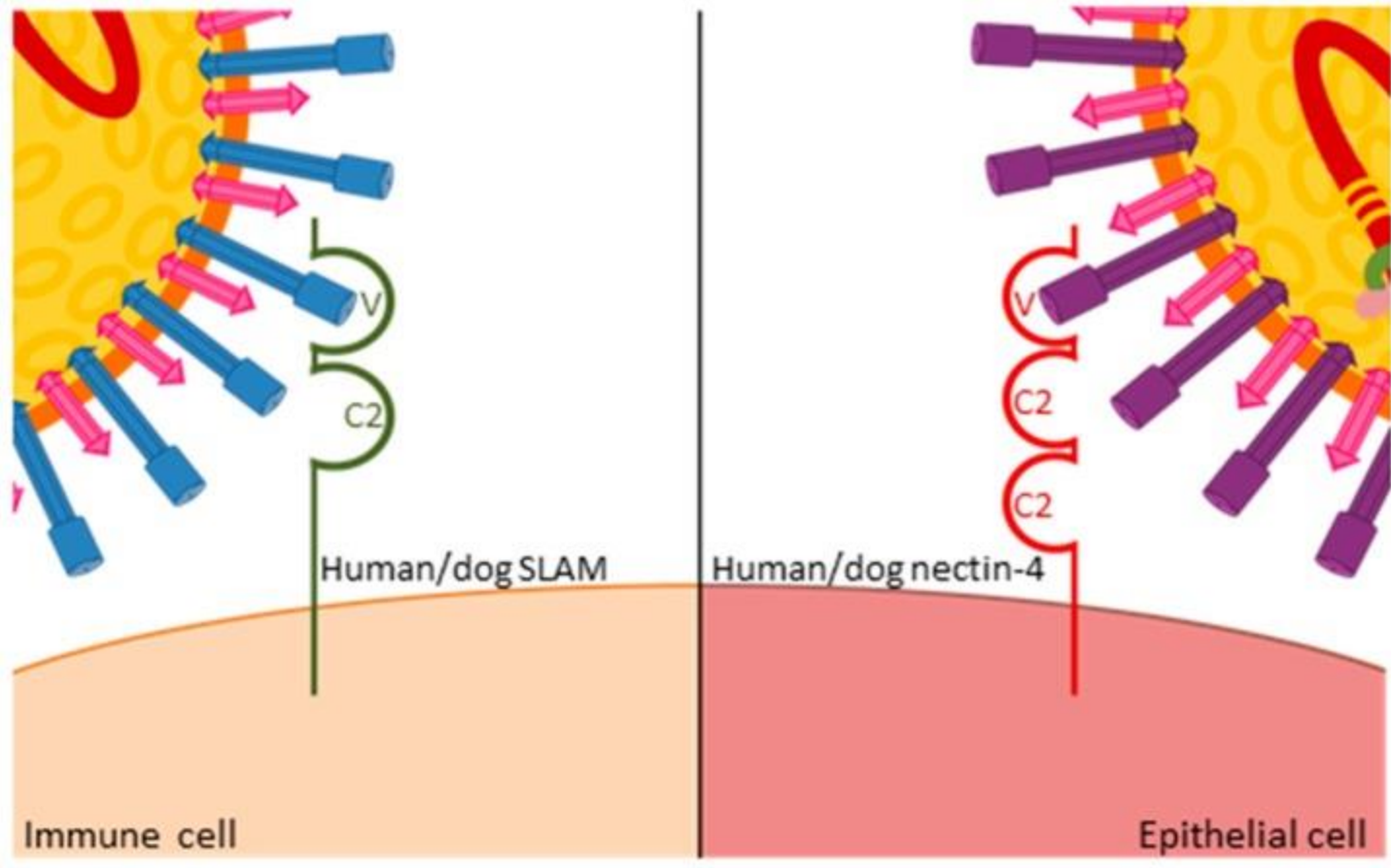
• Resident immune cells in the RT are the initial target

- Local immune organs
- **Systemic dissemination: first febrile peak 3-6 gg pi**
- This stage of the infection is critically dependent on the interaction of the virus with the immune cell receptor CD150 or SLAMF1
- **SLAMF1 has two extracellular domains, V and C2, interactions with H protein (variable domain, structural proximity)**
- Mutations in these H residues result in the missed infection of immune cells but replication in epithelial cells
- **Mutant viruses were able to cause replication in ferrets and protection but not systemic disease**
- Limited replication in epithelial cells close to the inoculation site likely occurs



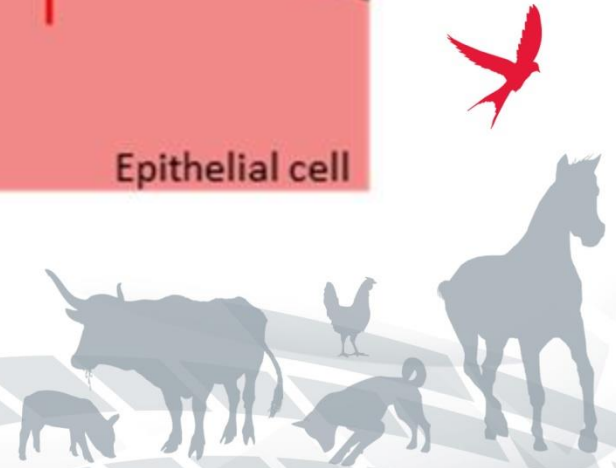


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Immune cell

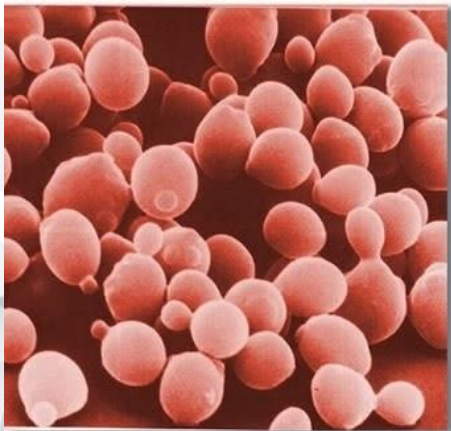
Epithelial cell



- **Efficiency of immune recognition by the respective host determines the extent of virus amplification in immune cells and thereby the level of spread to epithelial cells.**
- **In ferrets, which are unable to control wild type viruses, infection levels exceeding 80% of B or T cells can be observed in certain immune tissues, whereas levels below 10% are usually found in MeV-infected macaques**
- **Consequently, there is a massive infection of epithelial tissues in wild type virus-infected ferrets, leading to severe respiratory and gastro-intestinal signs of disease and death within two to five weeks whereas most primates develop only localized infection of epithelial cells and mild clinical signs**
- **SNC, 8-10 gg**
- **10-30 gg guarigione o replication**
- **20 gg Encefalomielite acuta**

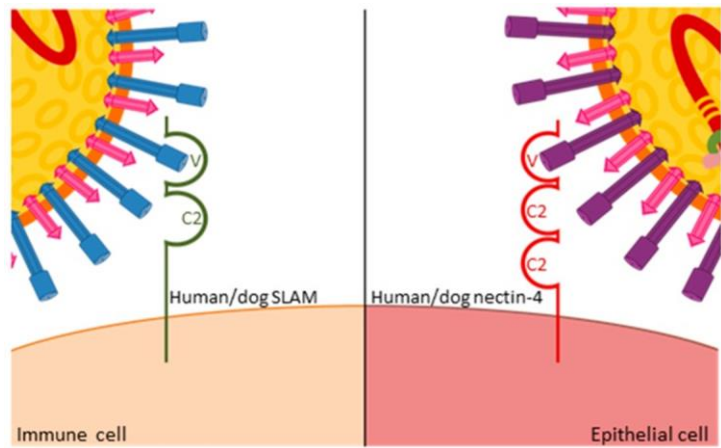


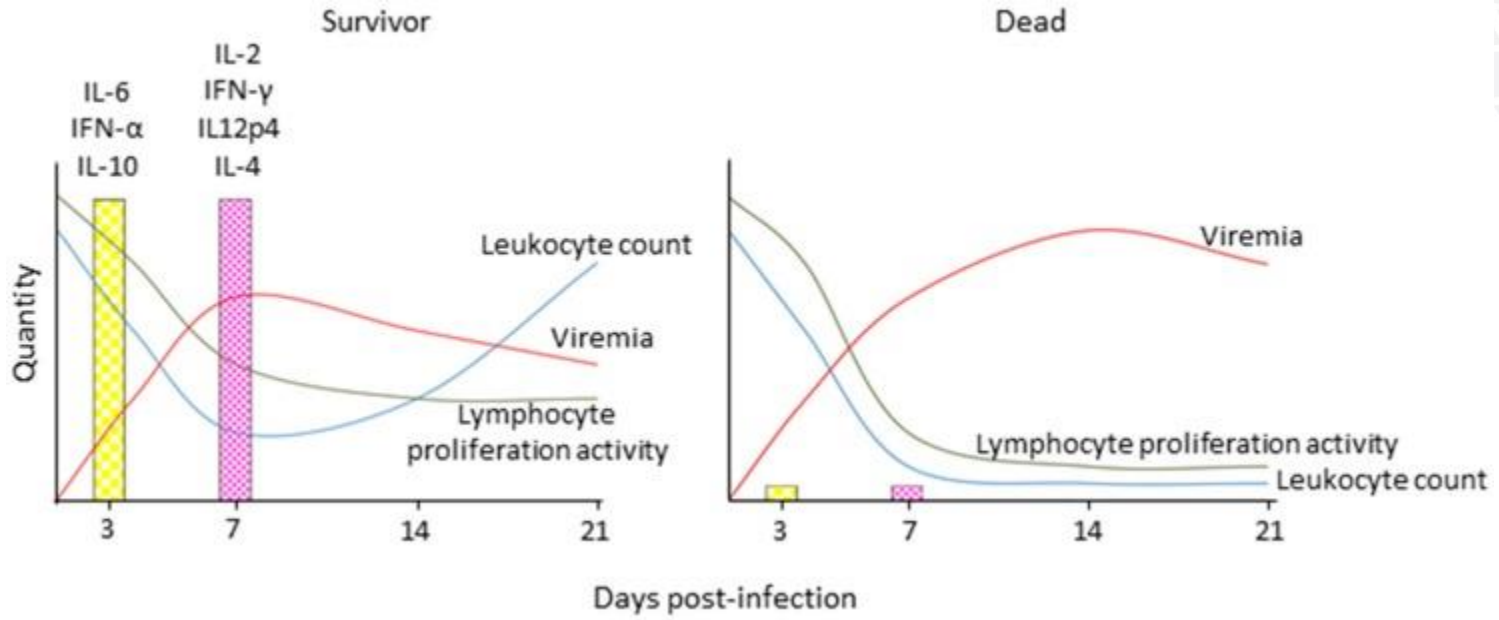
- 
- **The severe transient immunosuppression, which occurs at variable levels and can last from weeks to months after the resolution of the disease, is one of the hallmarks of morbillivirus infections.**
 - **Morbilliviruses additionally induce an anergy-like state in immune cells that prevents their ex vivo activation by non-specific stimuli and leads to the loss of delayed-type hypersensitivity responses**
 - **It is this long-term immunosuppression that leaves patients with an increased susceptibility to secondary infections such as pneumonia and gastroenteritis, which significantly contribute to MeV-associated morbidity and mortality**



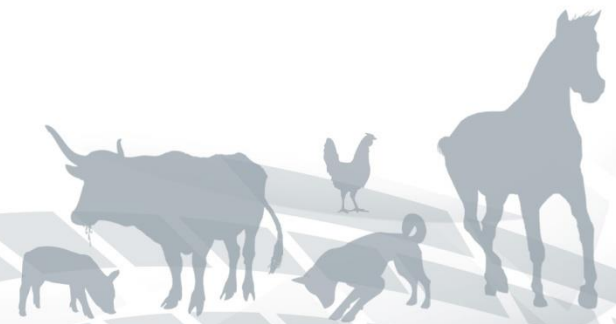


Immunosuppression is caused by immune cell infection while clinical signs, and likely also transmission, result from epithelial cell infection.





Schematic depiction of immune response profiles associated with different morbillivirus disease outcomes. Survivors display robust innate immune activation and experience transient immune suppression as indicated by a transient drop in leukocytes and lymphocyte proliferation activity upon non-specific stimulation, robust induction of innate and then adaptive cytokine responses, and control of cell associated viremia, while animals that succumb to the disease experience severe leukopenia and complete loss of lymphocyte proliferation activity, and are unable to activate innate immune responses and control the virus.



SINTOMI

Incubazione 1-4 settimane

1 ipertermia 3-4 gg pi

2 ipertermia dopo 7 gg pi

Acuta: epiteli respiratori, urogenitali, ghiandolari, cervello

Subacuta: RI tardiva, encefalite

Demielinizzazione acuta

Demielinizzazione cronica, dopo 6-7 settimane pi

Ipercheratosi cuscinetti plantari e tartufo (rara)

Ipoplasia smalto

Sintomi nervosi, epilessie, mioclonie, paresi.

Durano fino ad un mese e possono sparire con risposta immunitaria



DIAGNOSI IntraVitam!

Urine, tampone congiuntivale, sangue intero, siero (RT-PCR)

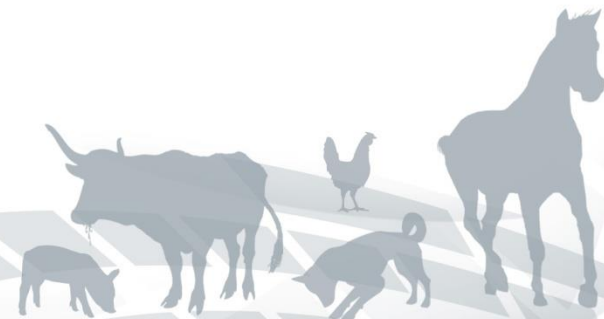
IF da tampone congiuntivale e buffy coat!

Ricerca IgM



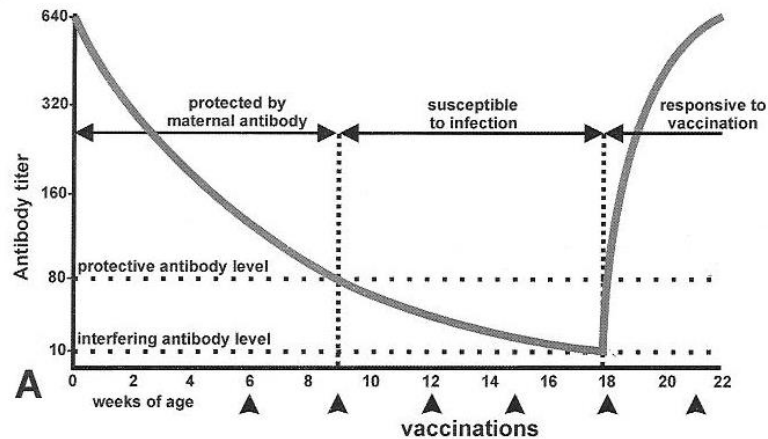
DIAGNOSI Post Mortem

Cervelletto, polmone, milza, vescica, linfonodi, RT-PCR, IHC



Profilassi

- Isolamento cani infetti
- Disinfezione ambienti
- Vaccini vivi attenuati, ottimi
- Interferenza MDA
- Gap immunologico tra la quinta ed ottava settimana (1:80-1:20)
- Emivita MDA 9 giorni



MDA ostacolano immunizzazione:

- **75% cuccioli a 6 settimane**
- **25% cuccioli a 9 settimane**
- **<5% cuccioli a 12 settimane**



Cuccioli < 16 settimane:

- **3 dosi tra la sesta e la sedicesima settimana, ogni 3-4 settimane**

Cani > 16 settimane:

- **Due dosi a distanza di 3-4 settimane**
- **1 sola dose può andare bene**

Rivaccinazione ad 1 anno e poi ogni 3 anni





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Cimurro




made in nature, made in Italy





Publicazione 19/10/2011 Revisione N. 1

 **ISTITUTO ZOOPIROFILATTICO Sperimentale DELL'ABRUZZO E DEL MOLISE G. CAPORALE**
 Campo Boario - 64100 Teramo
 Telefono 0861 3027 - Fax 0861 30251
 www.izs-venezianet.it

NRG

DATA DEL PRELIEVO: ____/____/____

SISTEMA INFORMATIVO FAUNA SELVATICA

MODULO CONFERIMENTO CAMPIONI

SPECIE _____	1° CONFERIMENTO
MATERIALE CONFERITO	
<input type="checkbox"/> Carcassa <input type="checkbox"/> Rene <input type="checkbox"/> Polmone <input type="checkbox"/> Muscolo <input type="checkbox"/> Milza <input type="checkbox"/> Cuore <input type="checkbox"/> Fegato <input type="checkbox"/> Intestino <input type="checkbox"/> Sangue <input type="checkbox"/> Siero <input type="checkbox"/> Contenuto gastrico <input type="checkbox"/> Altro _____ <input type="checkbox"/> Feci <input type="checkbox"/> Organi interni	<input type="checkbox"/> 1° ambiente <input type="checkbox"/> Refrigerazione <input type="checkbox"/> Congelamento
PROVENIENZA	RICHIEDENTE
<input type="checkbox"/> Vita libera <input type="checkbox"/> Area faunistica <input type="checkbox"/> CRAS <input type="checkbox"/> Altro _____ <input type="checkbox"/> Allevamento <input type="checkbox"/> Azienda agri-turistico-venatoria <input type="checkbox"/> Azienda turistico-venatoria	Ente/ _____ Indirizzo _____ Veterinario referente _____ Telefono _____ Email _____ Presente alla necropsia <input type="checkbox"/> SI <input type="checkbox"/> NO
Codice azienda: □□□ □□ □□□□	
Maf/razione _____	
Comune _____	
Provincia _____	
LUOGO DEL RITROVAMENTO	
PROVINCIA _____ COMUNE _____ LOCALITÀ _____ COORDINATE GEOGRAFICHE IN GRADI DECIMALI (WGS 84 LAT/LONG): LATITUDINE □□.□□□□□ LONGITUDINE □□.□□□□□ oppure COORDINATE GEOGRAFICHE IN FORMATO SESSAGESIMALE (GG° MM' SS"): LATITUDINE □□° □□' □□" LONGITUDINE □□° □□' □□"	
ANAMNESI _____	
IDENTIFICAZIONE ANIMALE	FOTO IN ALLEGATO <input type="checkbox"/> SI
SESSO: <input type="checkbox"/> M <input type="checkbox"/> F <input type="checkbox"/> NON IDENTIFICATO ETÀ: _____ (anni) IDENTIFICATIVO (MICROCHIP, MARCA AURICOLARE, RADICOLARE, RIFERIMENTO - ENTE DI PROVENIENZA)? _____	

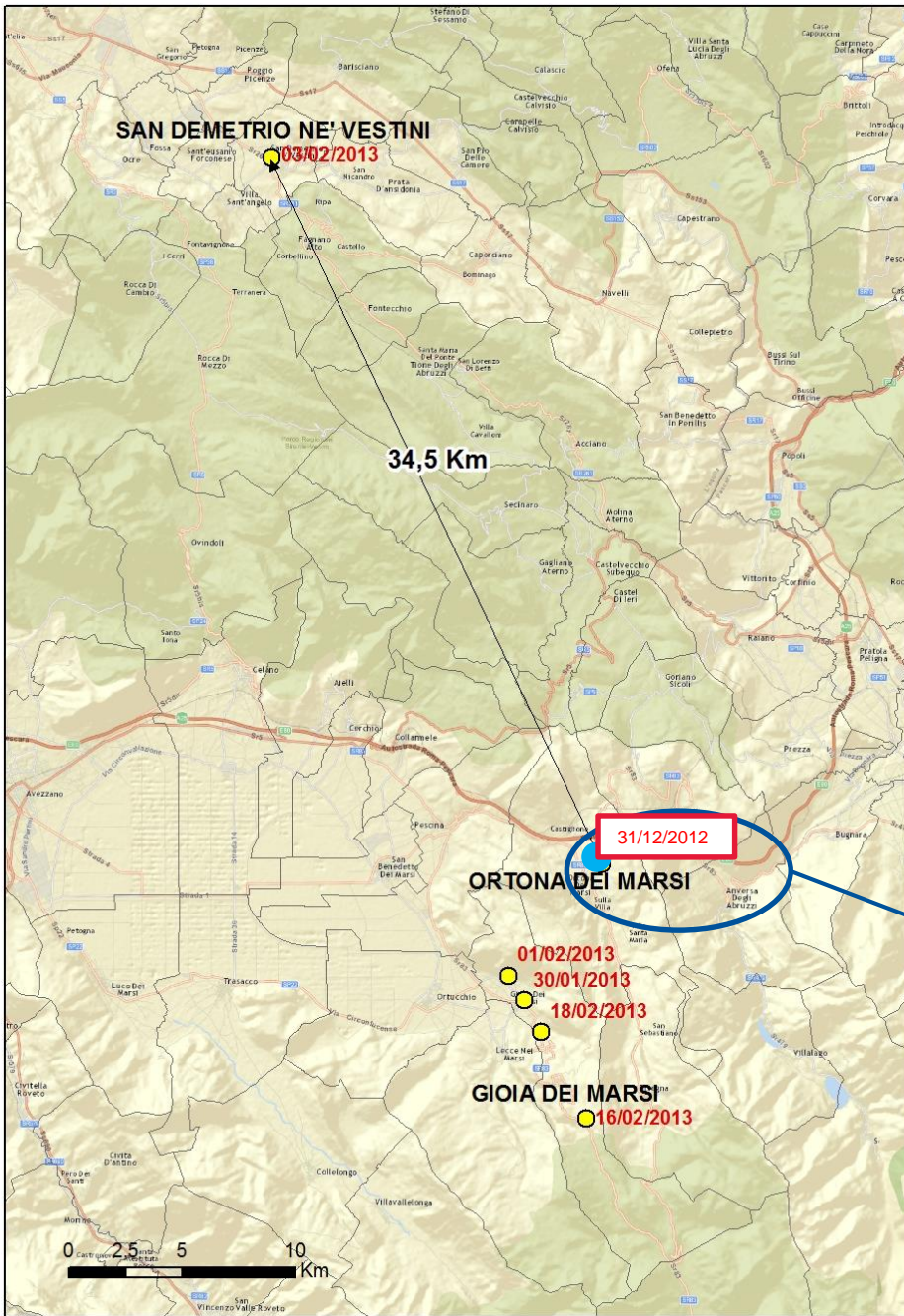
1/3

IZS dell'Abruzzo e del Molise FAUNA SELVATICA

CARNIVORI SELVATICI		
Tipologia accertamento	Materiale da prelevare	Accertamento
Parassitologico	Feci	Endoparassiti
	Mucolo tibiale craniale	Strongili Broncopolmonari
Virologico		Trichinella
	Intestino tenue	Parvovirus Cane
	Polmone	Cimurro
	Fegato	Epatite infettiva

Dal 2010 in IZSAM esiste una raccolta sistematica di informazioni utili a successive analisi



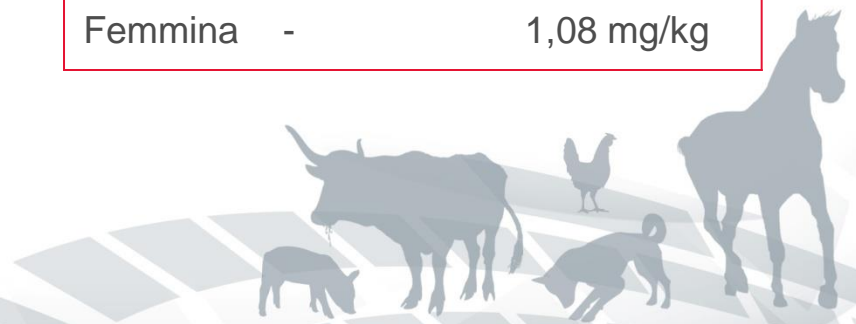


31 Dicembre 2012

Recuperati due lupi di età <1 anno ad Ortona dei Marsi
Sospetto avvelenamento

Rilievo di CARBOFURAN

id	Contenuto gastrico	Fegato
Maschio	1,24 mg/kg	1,83 mg/kg
Femmina	-	1,08 mg/kg





Ortona dei Marsi
31/12/2012

FEMMINA
Età < 1 anno
10,5 Kg

I lupi si presentavano
all'esame anatomo-
patologico: fortemente
defedati (dimensioni
corporee fortemente
ridotte per l'età)

e...



MASCHIO
Età < 1 anno
8,1 Kg

MASCHIO
Età < 1 anno
8,1 Kg

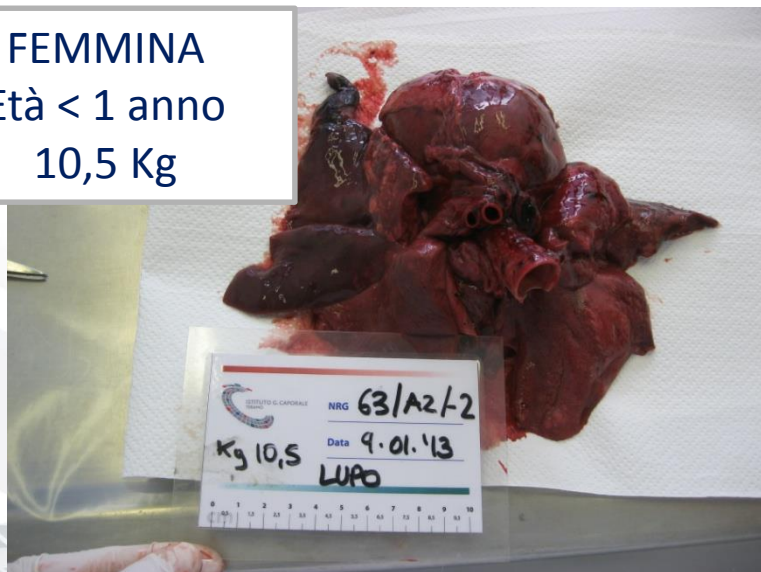


Broncopolmonite acuta caratterizzata da un denso infiltrato infiammatorio, costituito da granulociti neutrofili e macrofagi, che occupa le vie respiratorie profonde. Si osservano alcuni elementi sinciziali. Presenza di inclusi di probabile natura virale nei sincizi e nelle cellule epiteliali delle vie respiratorie.

Presenza di cellule immunoreattive nei confronti del virus del cimurro canino.

Encefalite caratterizzata da manicotti linfocitari perivasali.

FEMMINA
Età < 1 anno
10,5 Kg

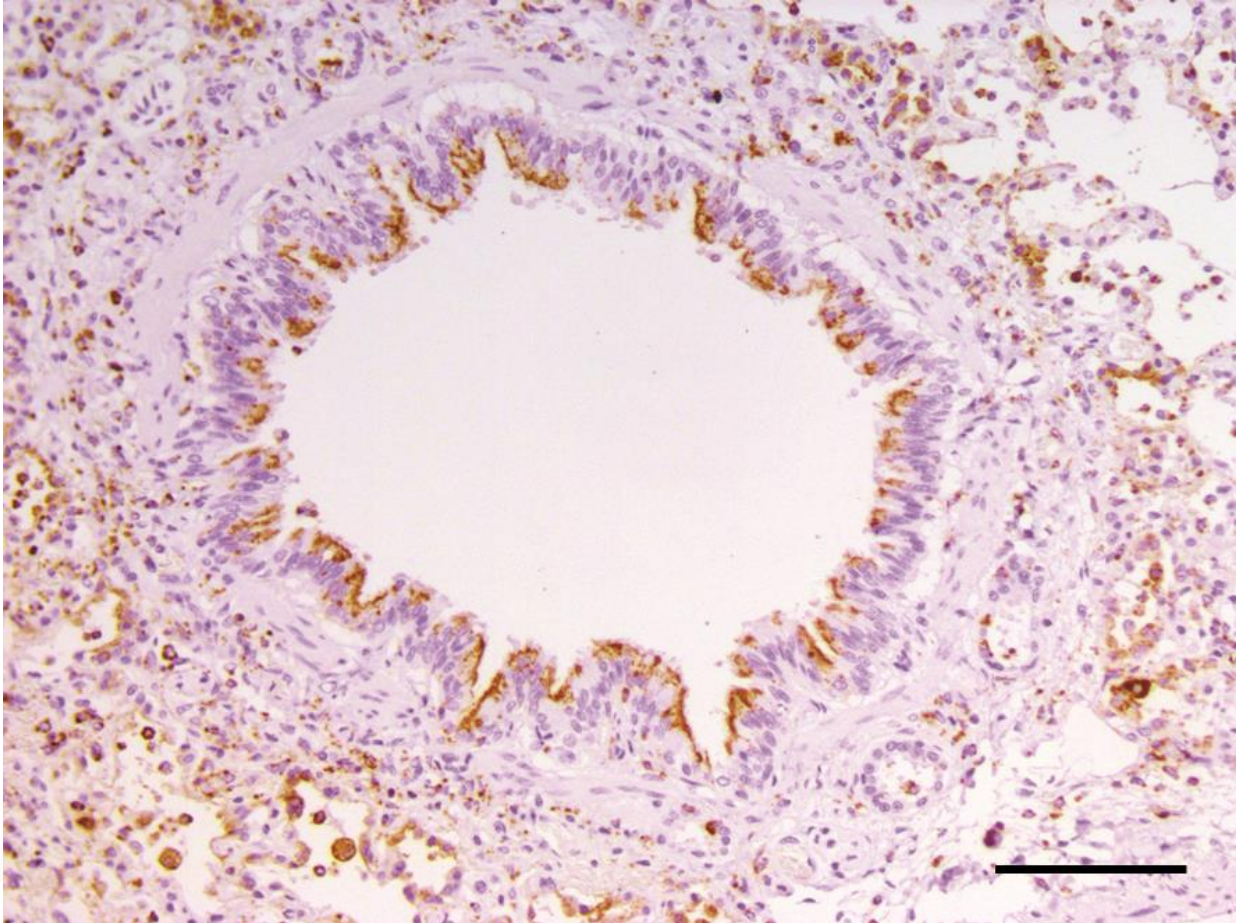


Ortona dei Marsi
31/12/2012





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CASI CIMURRO LUPI 2013

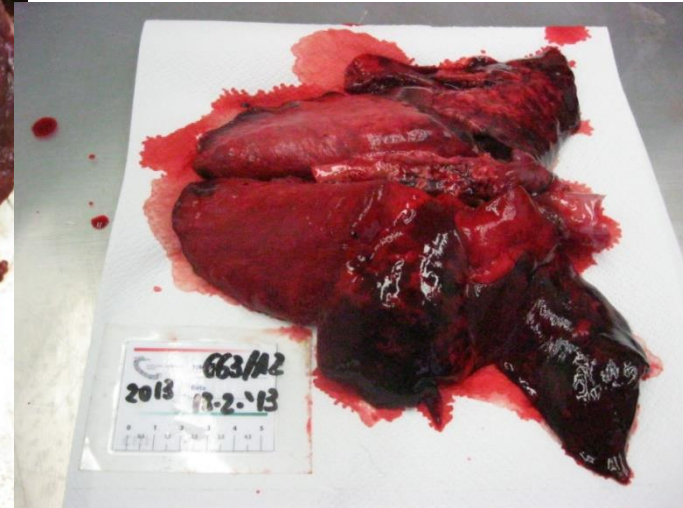


FEMMINA
Età < 1 anno
14,8 Kg

Broncopolmonite purulenta
con aree di emorragia.
Positività lieve e puntiforme per
antigene virale del cimurro

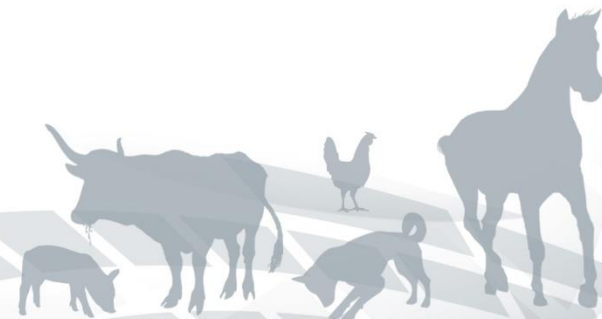
Meningite caratterizzata da
infiltrato infiammatorio misto

NRG 663/AZ/2013
San Demetrio
03/02/2013



Istopatologia ed immunoistochimica

- **Broncopolmonite caratterizzata da un denso infiltrato infiammatorio ricco di granulociti neutrofili e macrofagi alveolari. Aree di polmonite interstiziale con ispessimento dei setti alveolari e presenza di membrane ialine. Congestione vasale ed edema alveolare.(4016)**
- **Polmonite catarrale, presenza di inclusi intracitoplasmatici. Quadro riferibile a polmonite virale (2648)**
- **Encefalite e meningite**





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JOURNAL OF VIROLOGICAL METHODS 202 (2014) 64–68



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Contents lists available at ScienceDirect

Journal of Virological Methods

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Short communication

Whole genome sequence analysis of the arctic-lineage strain responsible for distemper in Italian wolves and dogs through a fast and robust next generation sequencing protocol



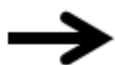
Maurilia Marcacci, Massimo Ancora, Iolanda Mangone, Liana Teodori, Daria Di Sabatino, Fabrizio De Massis, Cesare Camma', Giovanni Savini, Alessio Lorusso*

National Reference Centre for Urban Hygiene and Non-Epidemic Emergencies, Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale", 64100 Teramo, Italy





vero.DogSLAMtag cells



total RNA



random primers



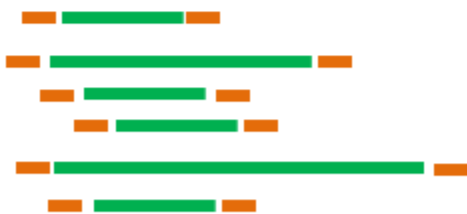
first strand cDNA synthesis



second strand cDNA synthesis



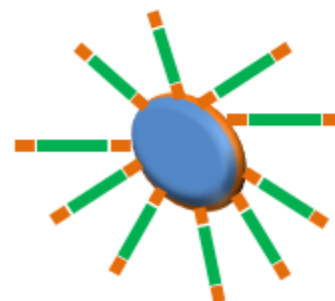
fragmentation



adapter ligation



size selection



beads hybridization and enrichment

Fig 1

America-1

Europe-WE/06-09

Europe-1

America-2

Europe-Wildlife

Asia-1

Asia-2

Arctic

South-African

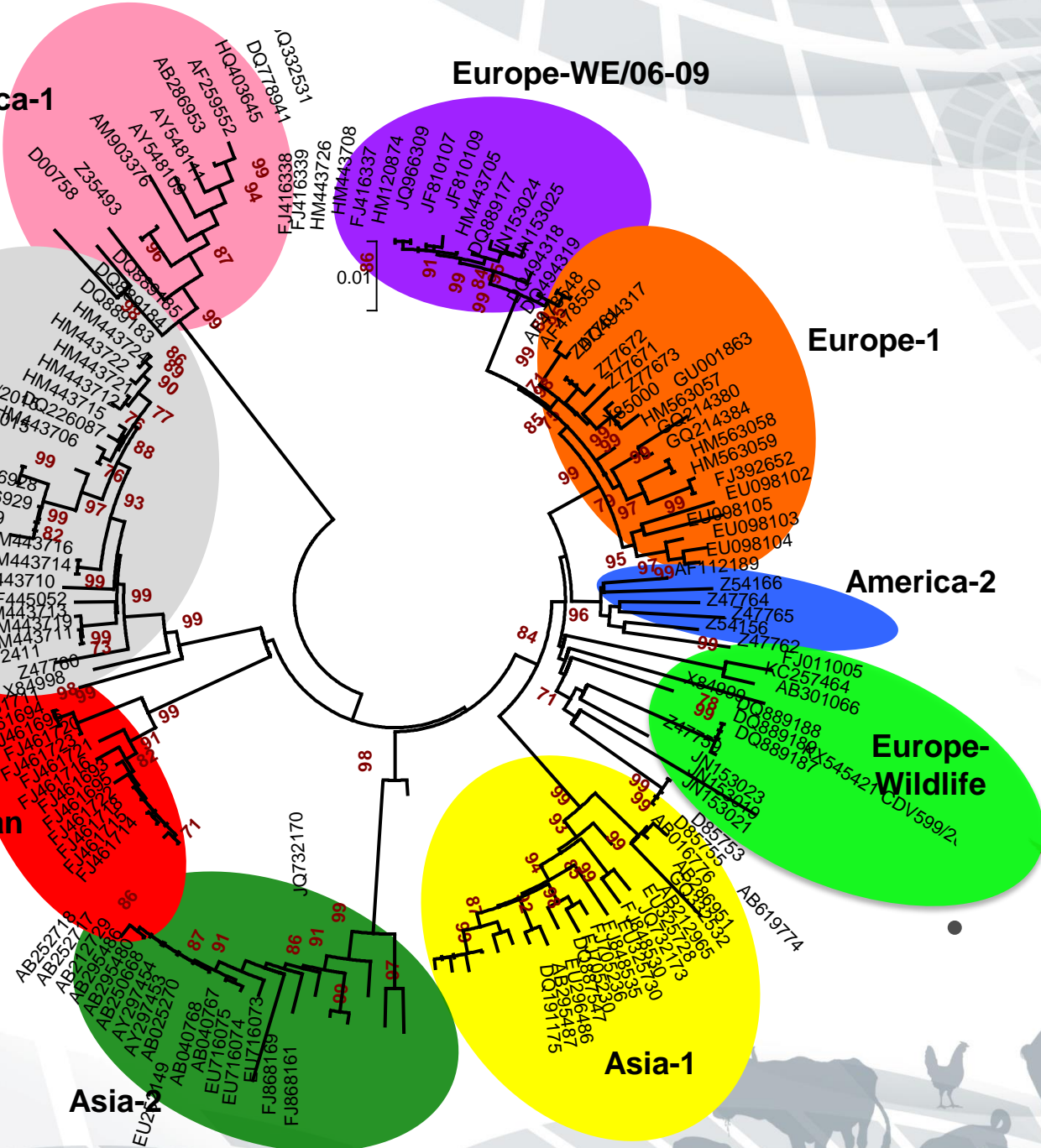
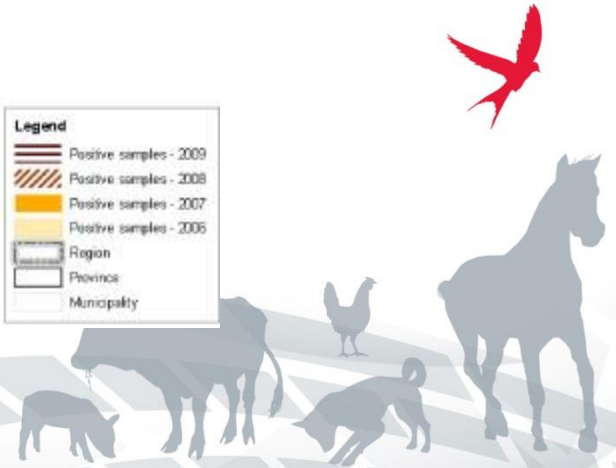
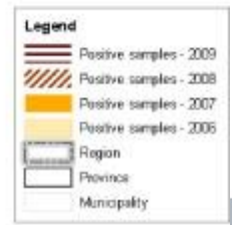
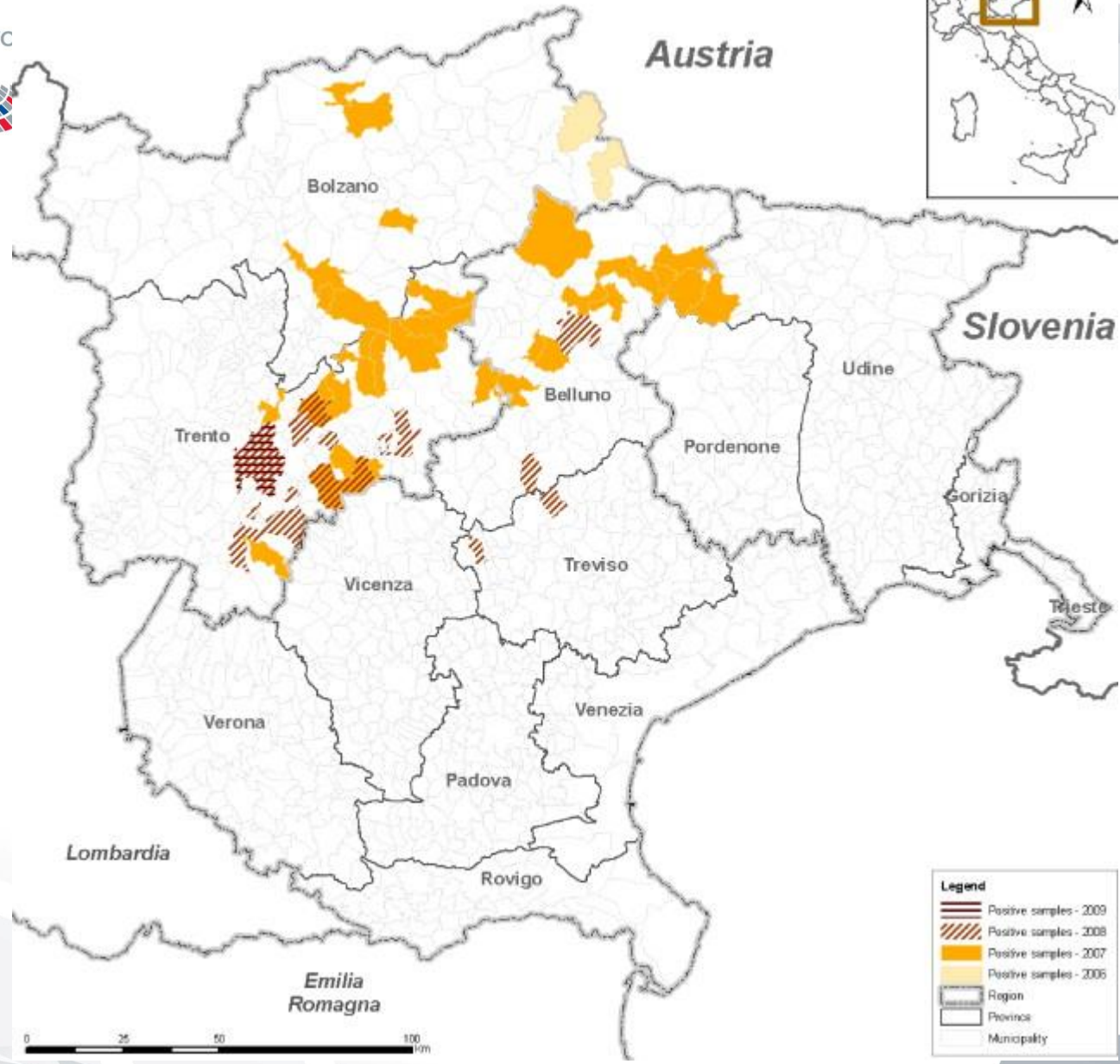


Fig 4



Arctic-like lineage

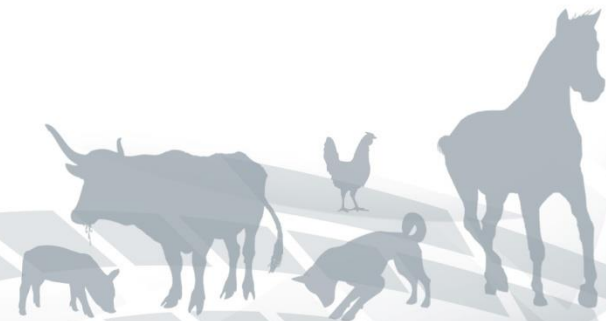
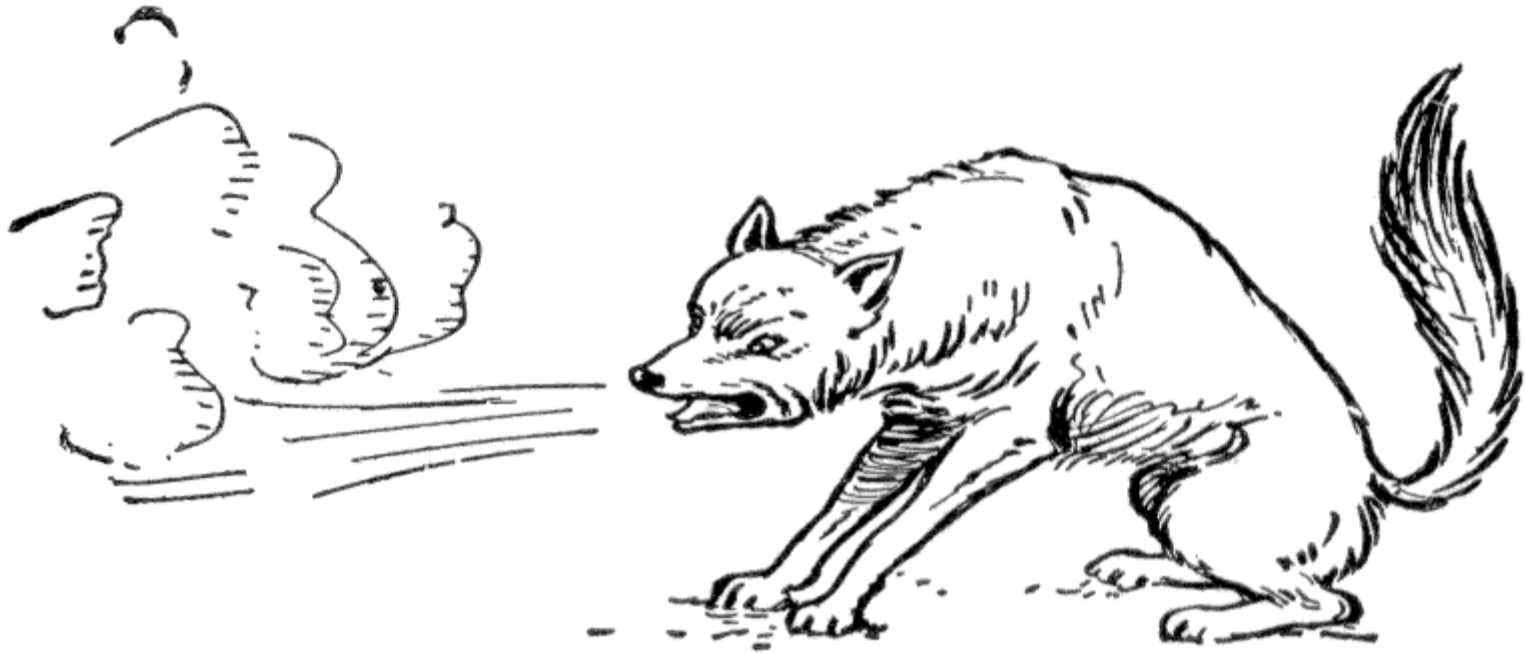
- Circolano nella popolazione canina domestica in Italia ed est Europa
- Prima segnalazione di epidemia nei selvatici
- In una sola volpe in Cina e' stato riscontrato un ceppo Arctic-like





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Cimurro..i primi casi clinici





Arctic Lineage-Canine Distemper Virus as a Cause of Death in Apennine Wolves (*Canis lupus*) in Italy

Daria Di Sabatino¹, Alessio Lorusso^{1*}, Cristina E. Di Francesco², Leonardo Gentile³, Vincenza Di Pirro³, Anna Lucia Bellacicco¹, Armando Giovannini¹, Gabriella Di Francesco¹, Giuseppe Marruchella¹, Fulvio Marsilio², Giovanni Savini¹

¹ Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy, ² Unit of Infectious Diseases, Faculty of Veterinary Medicine of Teramo, Teramo, Italy, ³ Veterinary Services, National Park of Abruzzi, Lazio and Molise, Pescasseroli (AQ), Italy



Fine Gennaio 201: Recuperati 3 lupi vivi



Maschio, 6-7 mesi di età

Femmina, 6-7 mesi di età

Maschio, 4-5 anni di età

Al ritrovamento:

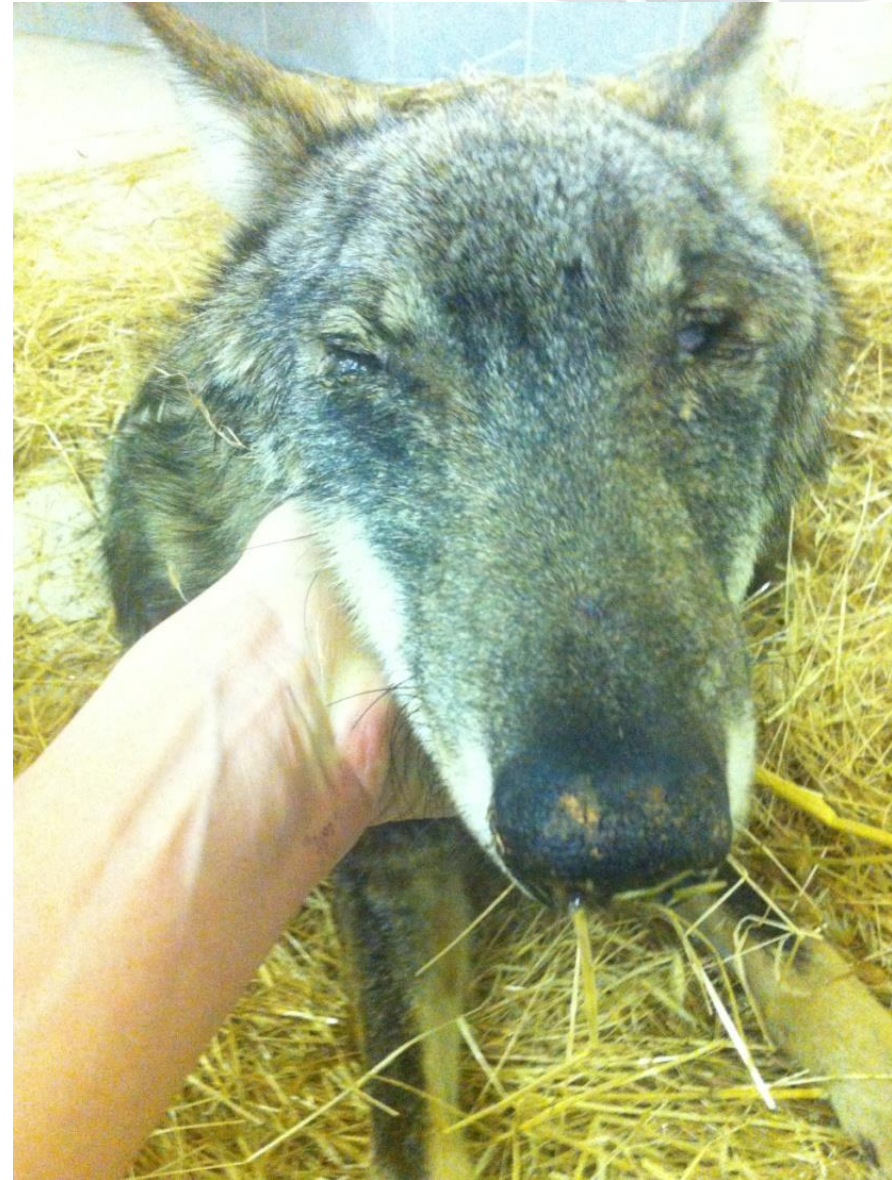
- Iporeattiva
- Denutrita
- Incoordinazione motoria
- Diarrea con tracce di sangue vivo
- Parametri: TR 37,5; FR 16; FC 60

Progressivo peggioramento con:

- Crisi convulsive ripetute
- Scolo nasale bilaterale con abbondante muco giallastro e denso
- Grave congiuntivite bilaterale
- Aggravamento condizioni generali e coma
- Decesso

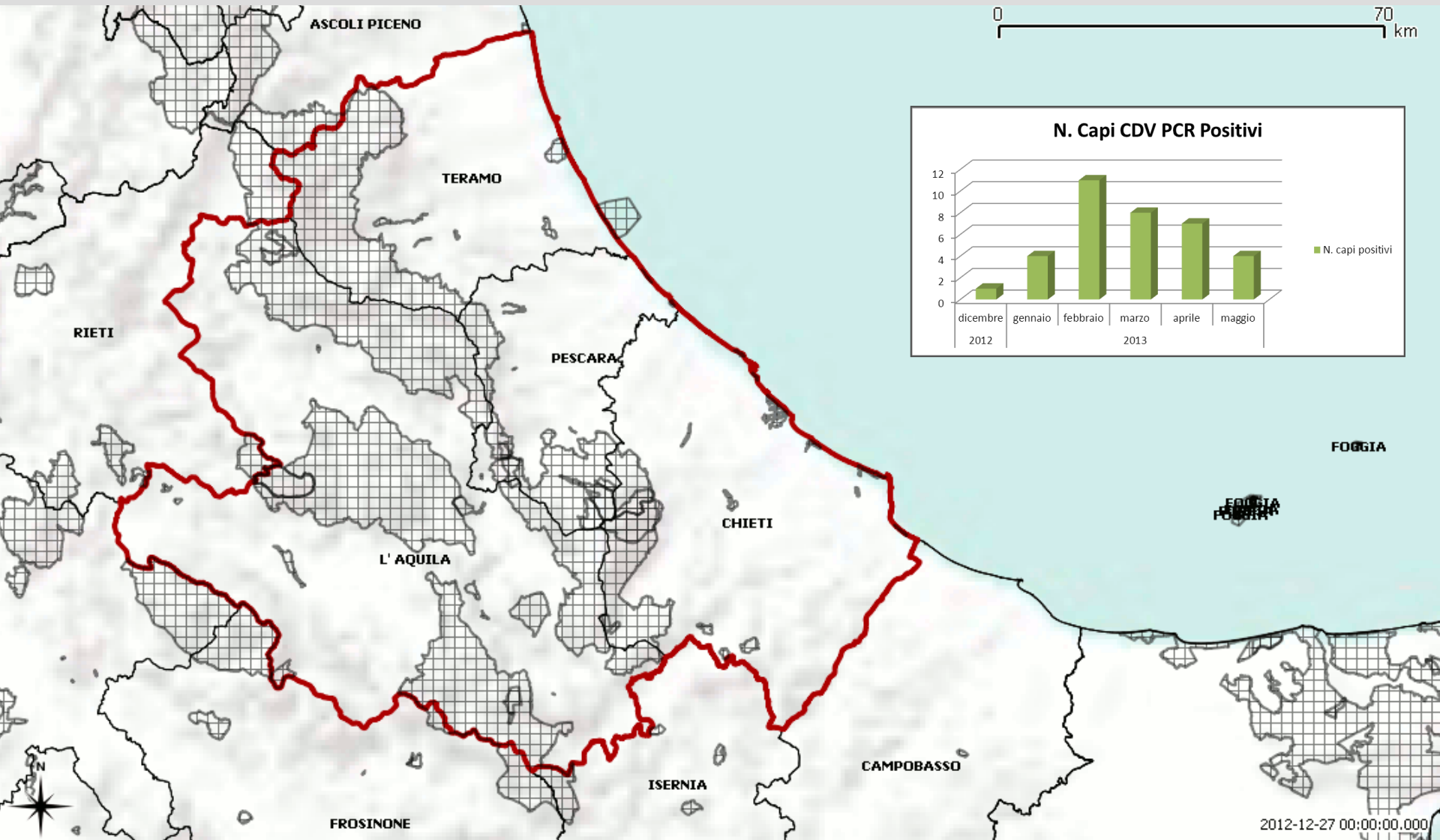


Primi casi con sintomi clinici

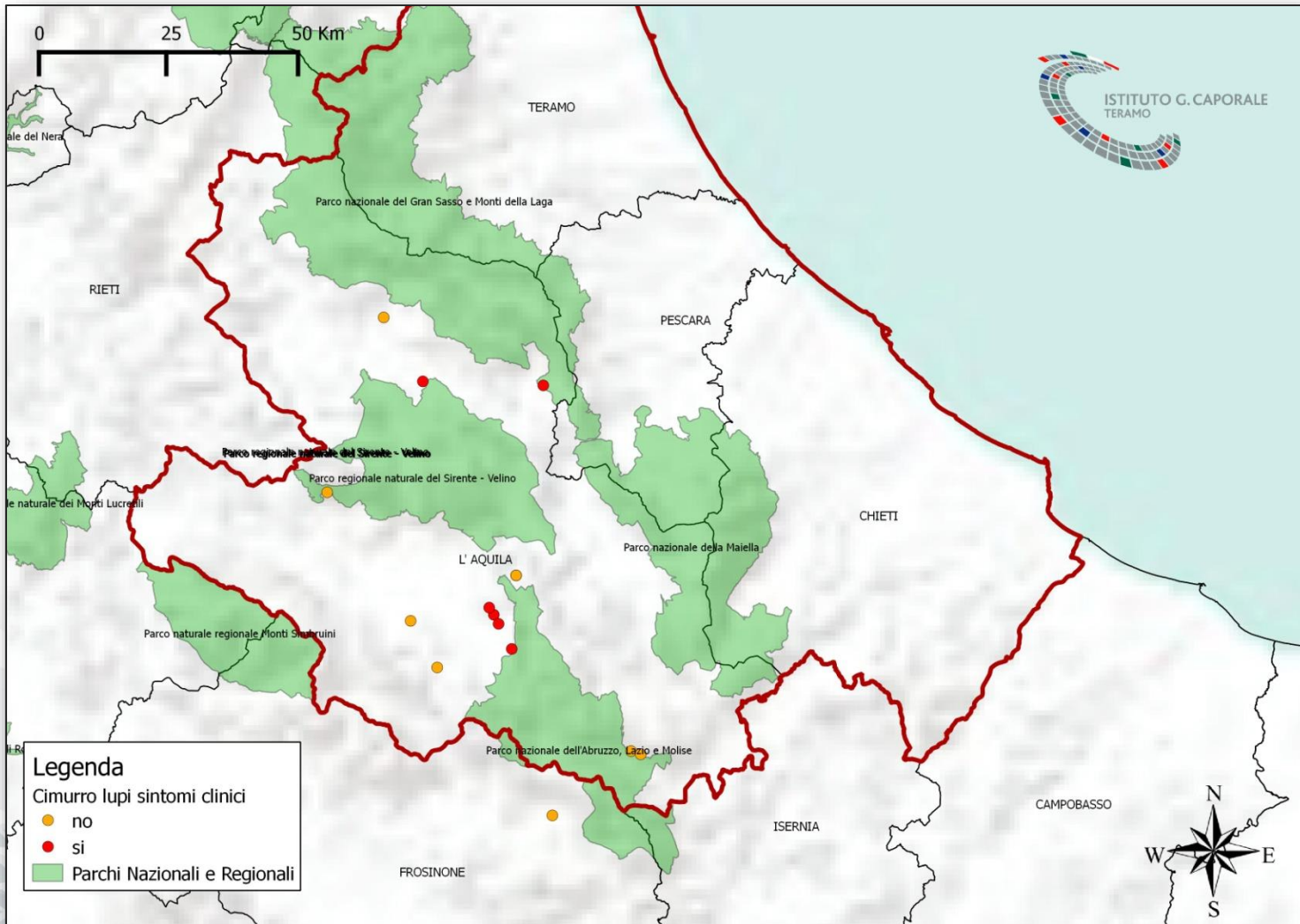


PROGRESSIONE TEMPORALE CASI CIMURRO CARNIVORI SELVATICI

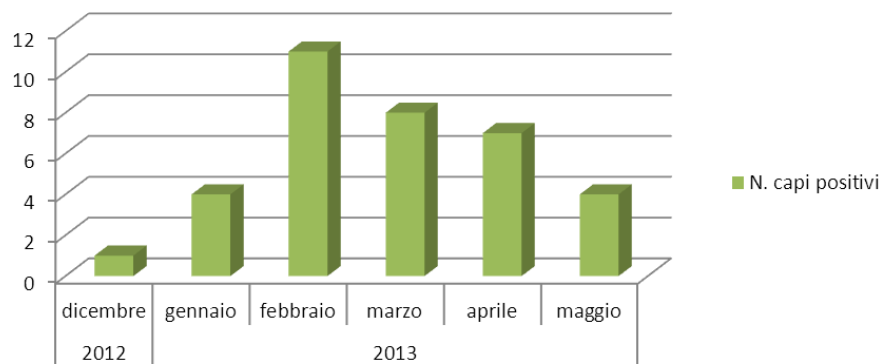
Primo caso 31/12/2012 ultimo caso 29/05/2013



CASI CLINICI CIMURRO LUPI 2013

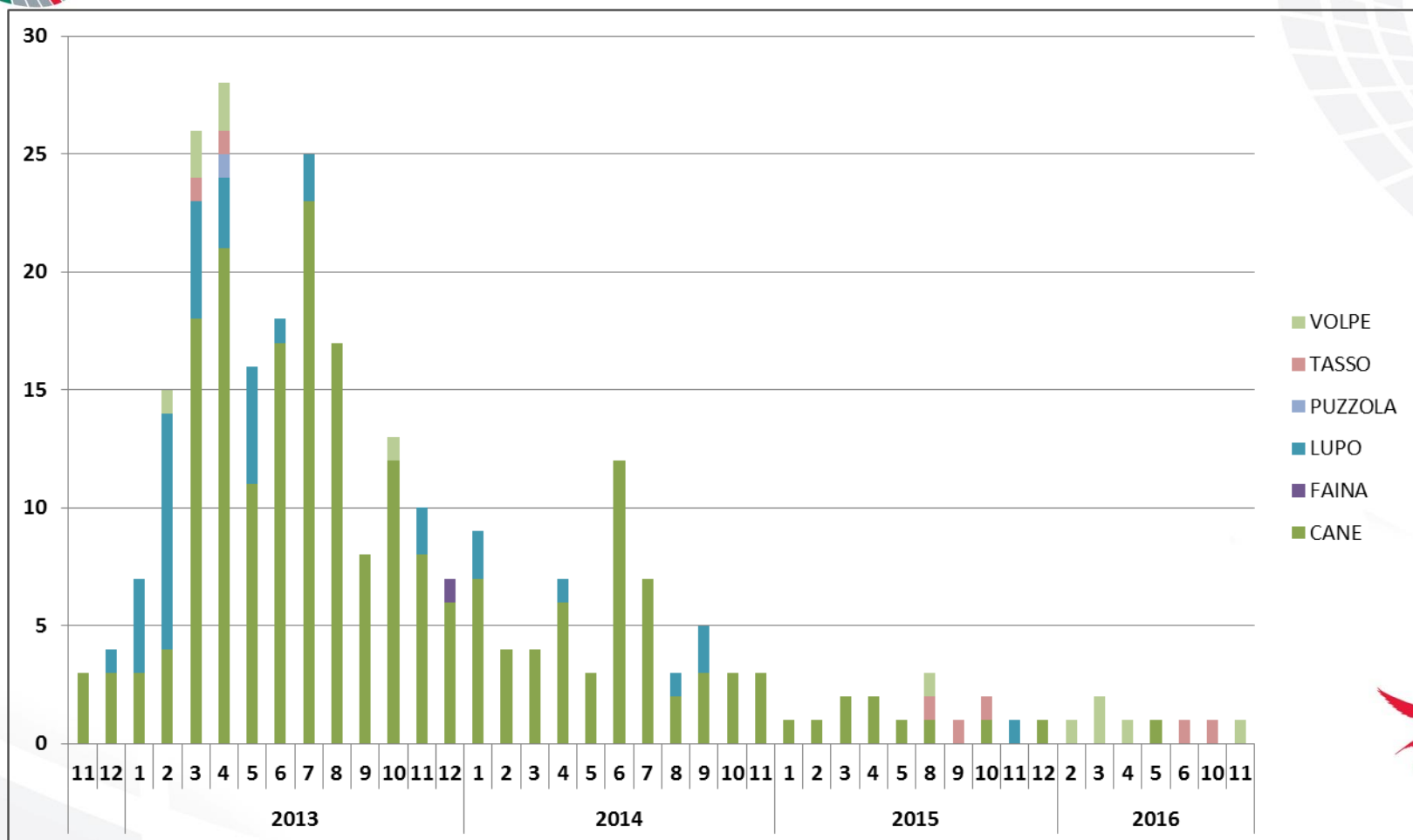


N. Capi CDV PCR Positivi



Provincia	Comune	N. capi Positivi
ASCOLI PICENO	ARQUATA DEL TRONTO	1
FROSINONE	SETTEFRATI	1
ISERNIA	CASTEL SAN VINCENZO	2
L' AQUILA	AVEZZANO	1
	BARREA	2
	CAPESTRANO	1
	CELANO	1
	CIVITELLA ALFEDENA	1
	COLLELONGO	1
	GIOIA DEI MARSII	4
	L'AQUILA	2
	MASSA D'ALBE	2
	OPI	1
	ORTONA DEI MARSII	2
	OVINDOLI	1
	PESCASSEROLI	4
	ROCCA DI MEZZO	1
	SAN DEMETRIO NE' VESTINI	1
	SULMONA	2
	TIONE DEGLI ABRUZZI	1
	TRASACCO	1
	VILLAVALLELONGA	1
TERAMO	TERAMO	1
Totale		35





L'epidemia di cimurro nei selvatici si è conclusa nella primavera-estate del 2013, solo casi sporadici sono stati confermati nei mesi e negli anni a seguire

L'epidemia di cimurro nei selvatici del 2013, ha interessato prevalentemente soggetti giovani (di cui il 40% inferiore ad 1 anno)

Specie	2012	2013			2014		2015		2016		Totale
	Inferiore ad 1 anno	adulto	Inferiore ad 1 anno	sub-adulto	adulto	Inferiore ad 1 anno	adulto	Inferiore ad 1 anno	adulto	sub-adulto	
LUPO	2	8	13	8	3	2		1			37
LUPO-IBRIDO					1						1
PUZZOLA		1									1
TASSO		2					2	1		1	6
VOLPE		3	1	1				1	3	1	10
Totale	2	14	14	9	4	2	2	3	3	2	55

Il rilievo di casi in soggetti giovani negli anni successivi indica una circolazione residua del virus

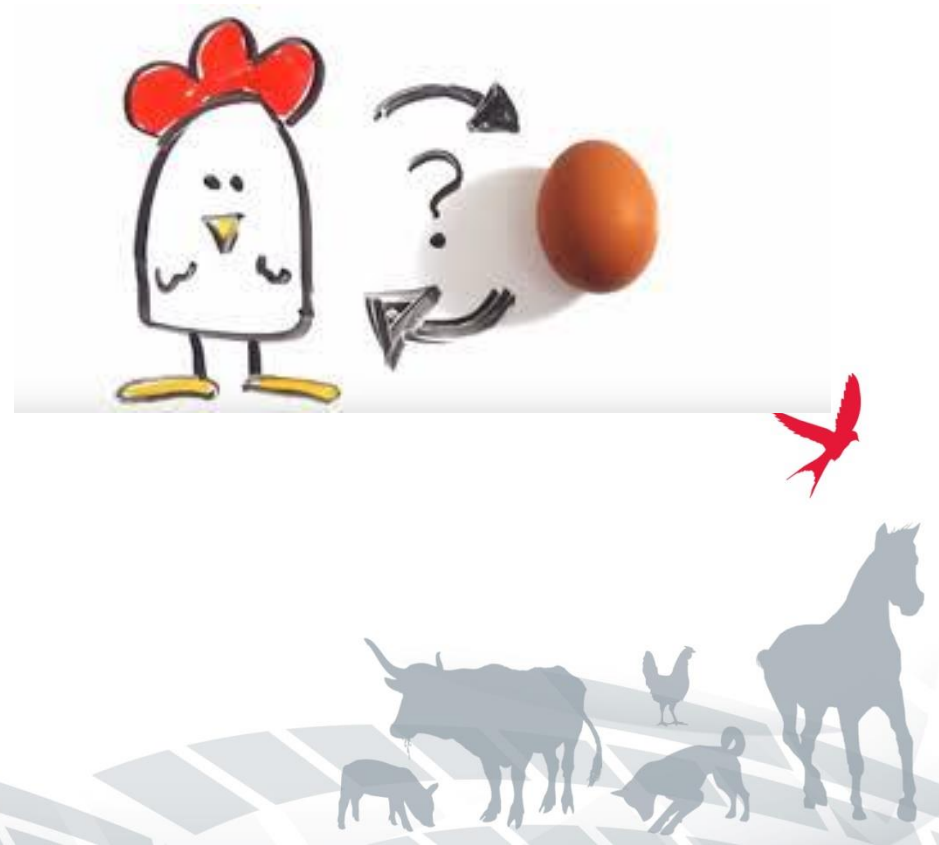


VACCINAZIONE !



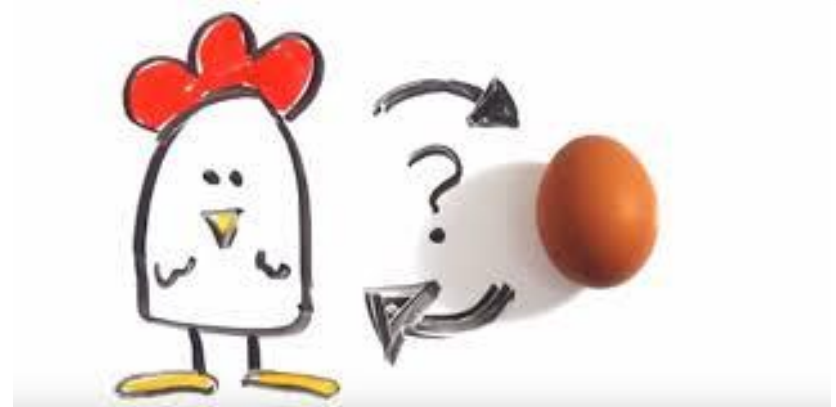
CIMURRO- oggi

- L'epidemia dei cimurro nei selvatici del 2013 è ascrivibile con molta probabilità all'introduzione del virus del ceppo artico dalla popolazione canina a quella selvatica...
- Ma dov'è il virus oggi?



CIMURRO- oggi

- La popolazione di lupi e/o di volpi è in grado di mantenere la circolazione virale in natura?
- In altre parole il virus del cimurro si è endemizzato nella popolazione selvatica?



Canine distemper spillover in domestic dogs from urban wildlife.

Kapil S¹, Yeary TJ.

CIMURRO- oggi

La **vaccinazione** dei cani a rischio di contatto con la popolazione selvatica rimane comunque la strategia chiave per:

- **Proteggere I cani** da eventuali spillo-over dalla fauna selvatica nel caso di endemia nei selvatici
- **Proteggere I carnivori selvatici** da eventuali nuovi ingressi del virus dalla popolazione canina





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Letter to the Editor

Canine distemper and endangered wildlife: Is it time for mandatory vaccination of dogs?

Keywords:
Distemper
Vaccination
Dogs
Wildlife

Infection by canine distemper virus (CDV) has been reported in all families of terrestrial carnivores as well as in some marine mammals. It causes a severe systemic and fatal disease [1]. In the last three decades, several outbreaks concerning endangered wildlife species were described worldwide and Italy as well experienced in the recent years severe CDV outbreaks in both domestic and sylvatic scenarios [2]. One of the last occurring CDV outbreaks has been reported in wolves in central Italy, in an area which hosts a critically endangered small population of brown bears (*Ursus arctos marsicanus*) [3]. CDV is a multi-host pathogen, and this characteristic assumes higher importance in the wildlife milieu. Interaction, immigration, feeding and mating are the main routes for sharing CDV between individuals of the same species. On the other hand, predators or scavengers of different species interact during feeding on the same animal or carcass. Endangered wild animals are threatened by multi-host pathogens in case of abundant sympatric species (domestic or wild) acting as reservoir, and with frequent interspecies interaction able to create a mixed host-species scenario that endures the maintenance of a persistent infection.

However, although CDV transmission dynamics and maintenance in wildlife are more complex than previously stated [4], undoubtedly the primary reservoir of CDV for wildlife are the infected domestic dogs. Dog population size and density provide indeed optimal conditions for maintaining a large and dense susceptible population which act as a reservoir for CDV with potentially catastrophic impact for the conservation of isolated wild populations. In some areas, mainly in spring and summer, when the number of wildlife juvenile individuals is higher, the scenario of overlap between the areas frequented by wildlife and by livestock guardian dogs (LGD) or stray dogs is not uncommon. In this scenario CDV transmission from dogs to wildlife has been well described in the African and European contexts [3,4]. Vaccine-based prophylaxis is the only effective way to keep clinical distemper disease under control in dogs. As massive vaccination of wild animals is

not feasible, the control of the interface between domestic dogs and wildlife has a crucial role to prevent the spread of the virus in critical areas in which endangered species are hosted. Massive vaccination of domestic dog in "strategic" areas must be considered as valid preventive measures to protect rare and susceptible wild species. Vaccine coverage of 95% is needed to control distemper in dogs [5]. Therefore legislating for mandatory CDV vaccination, prioritizing for domestic and LGD dogs has to be considered nearby parks or natural areas. Long-lasting immunity for CDV, which can be reached by a single shot of vaccine, needs to be extended to domestic dogs and to be continuous and widespread. Indeed, CDV epidemics are yet common in dogs and poor vaccination coverage is the main reason explaining this phenomenon.

Conflict of interest

The authors disclose any actual or potential conflict of interest including any financial, personal or other relationships that could inappropriately influence, or be perceived to influence, their work.

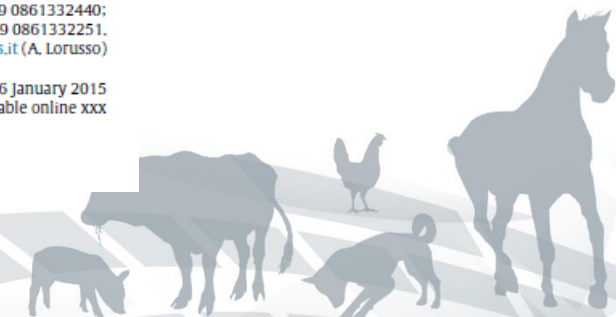
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26 January 2015
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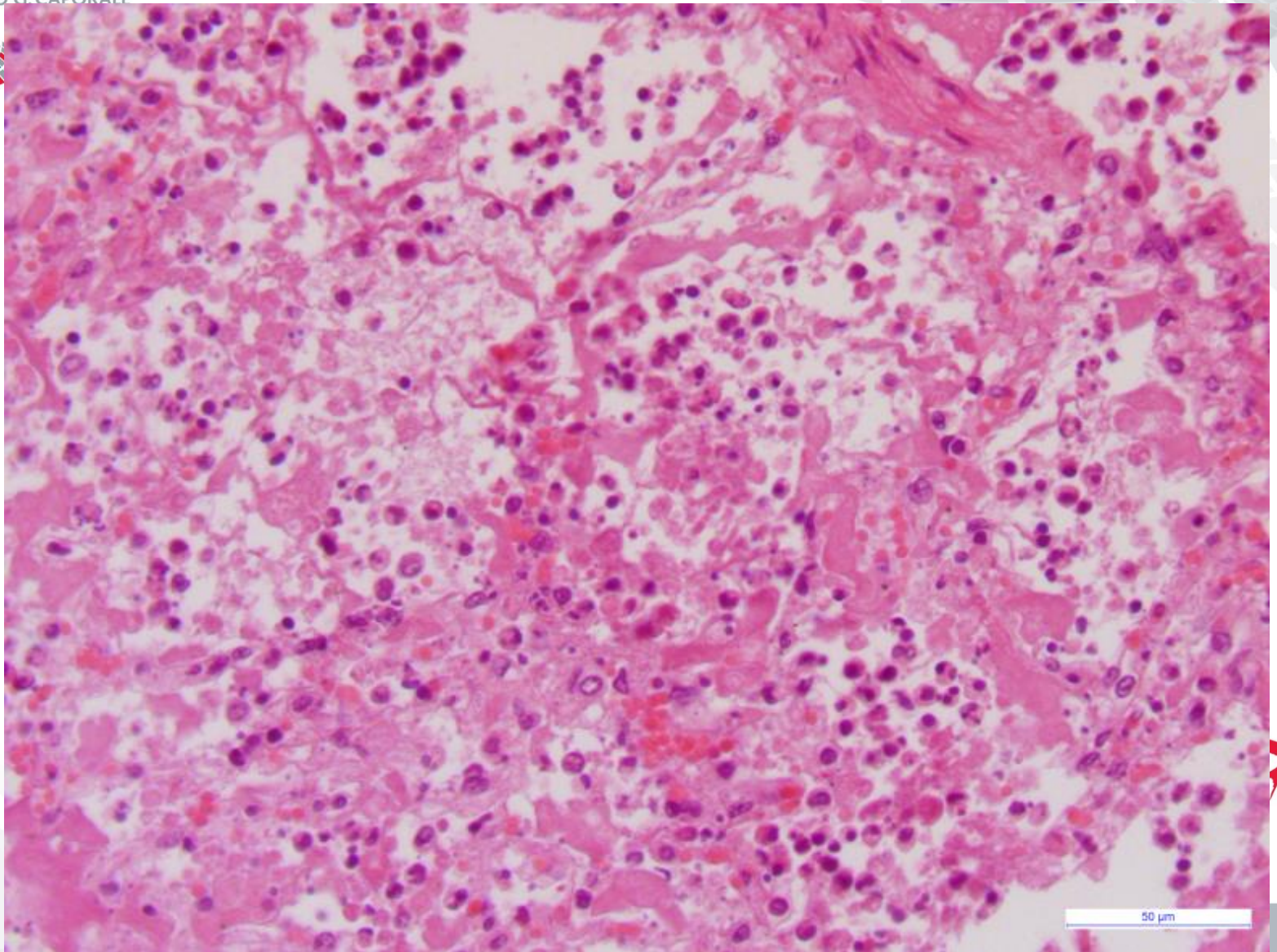




ISTITUTO G. CAPORALE
TERAMO

2015





50 μm

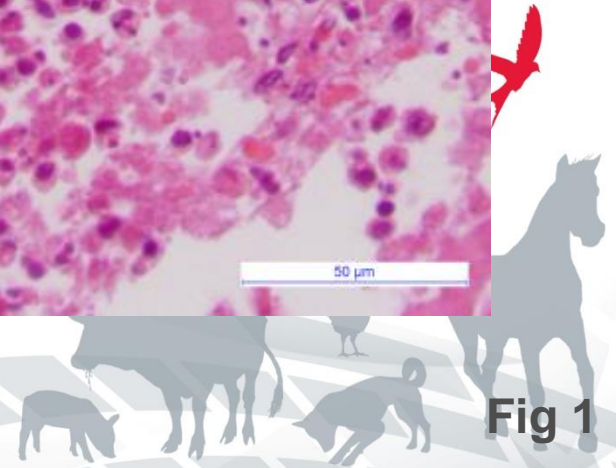
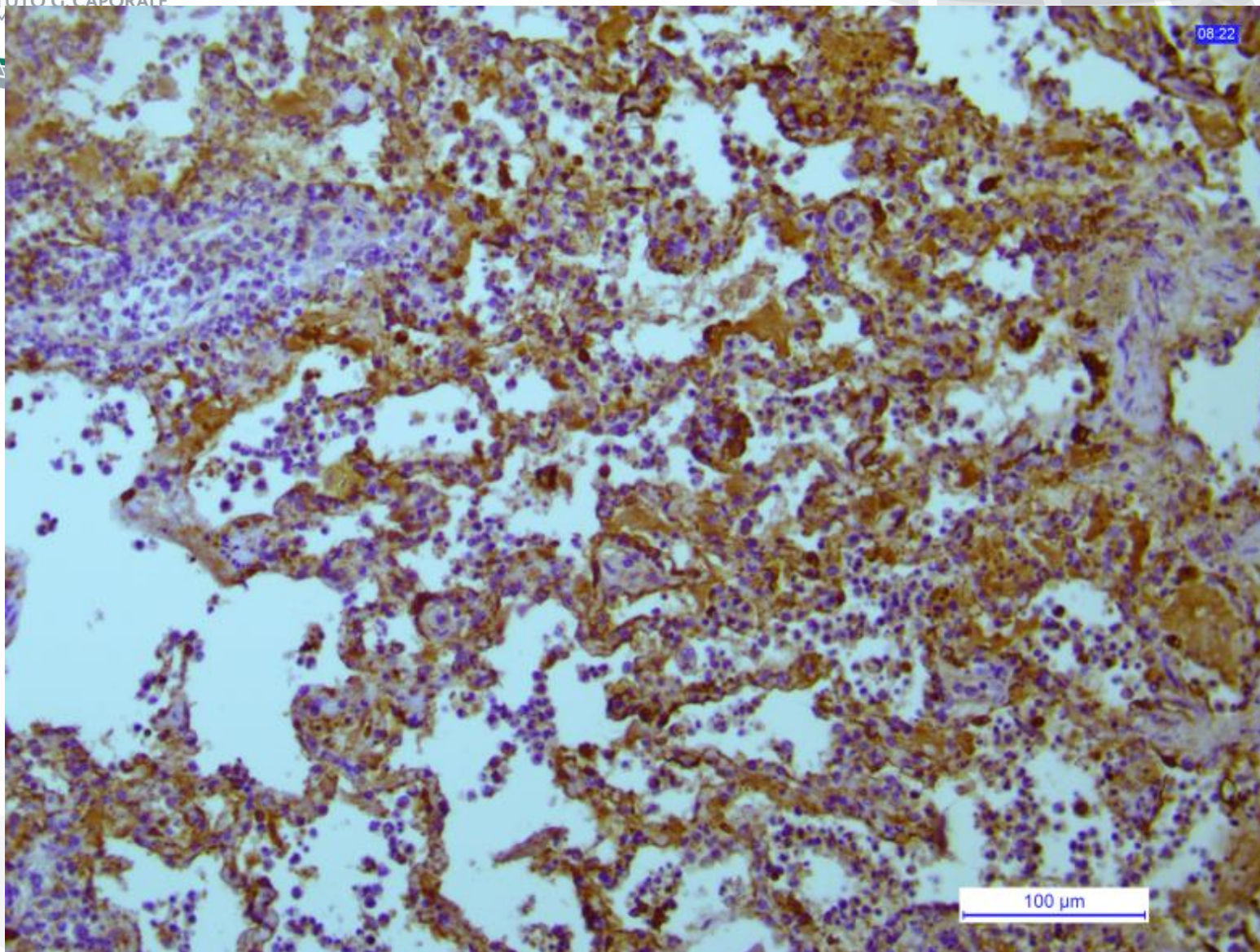


Fig 1



100 μ m



Fig 2A

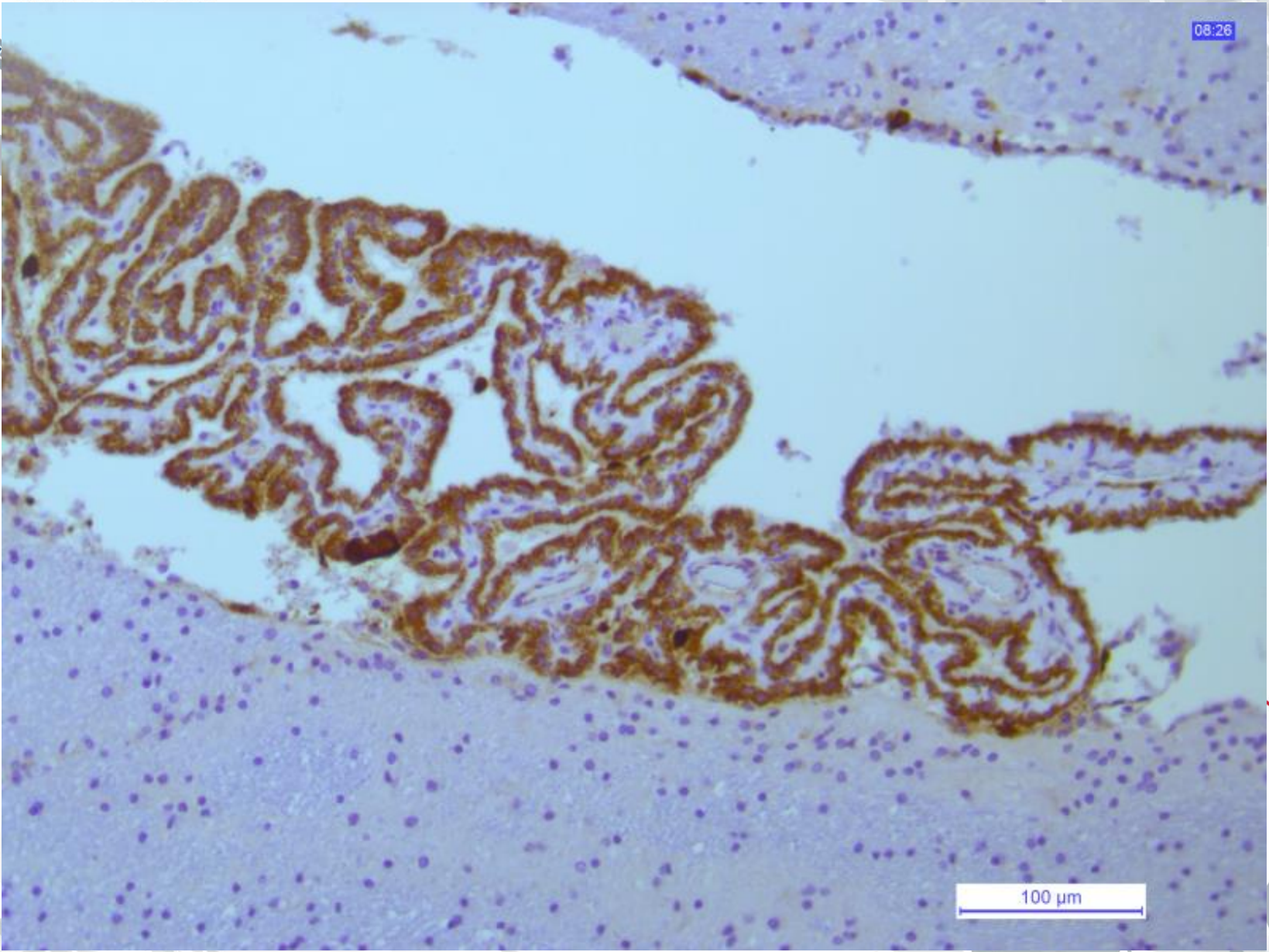
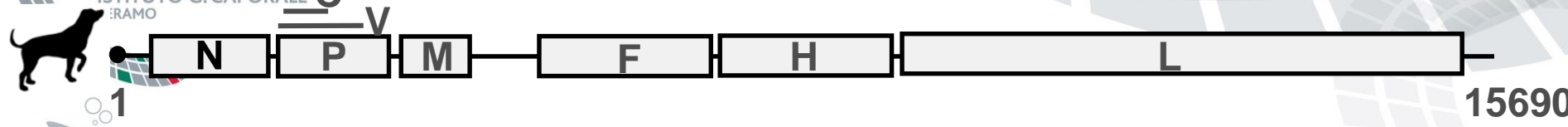


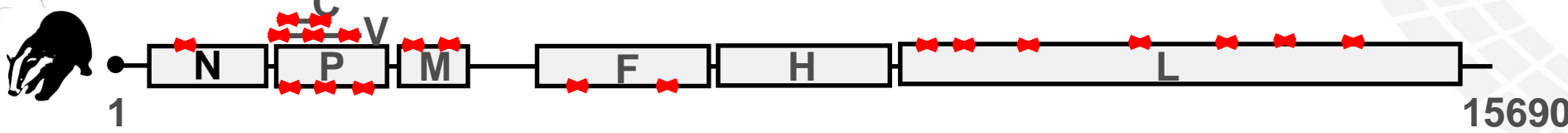
Fig 2B

CDV2784/2013

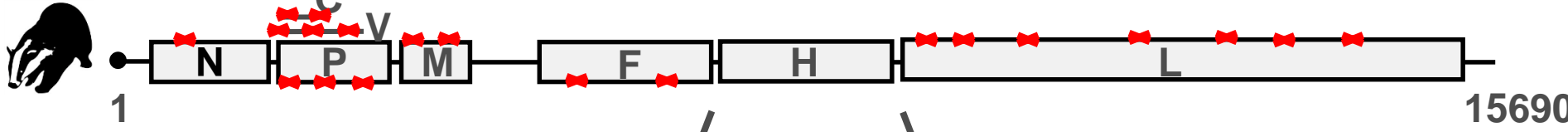
ISTITUTO G. CAPORALE
RAMO **C**



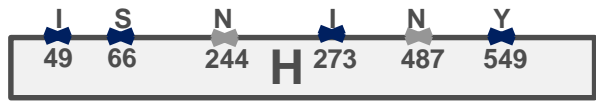
CDV11956/2015



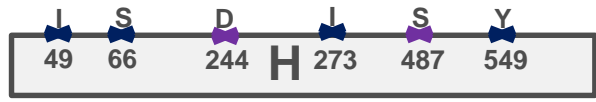
CDV12254/2015



CDV2784/2013



Wa-CDV2013



CDV11956/2015, CDV12254/2015

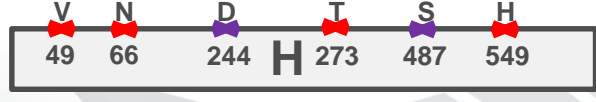


Fig 3

number of positive animals



Dog
Wildlife



Fig 5



ISTITUTO G. CAPORALE
TERAMO

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Research paper

Lethal distemper in badgers (*Meles meles*) following epidemic in dogs and wolves



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