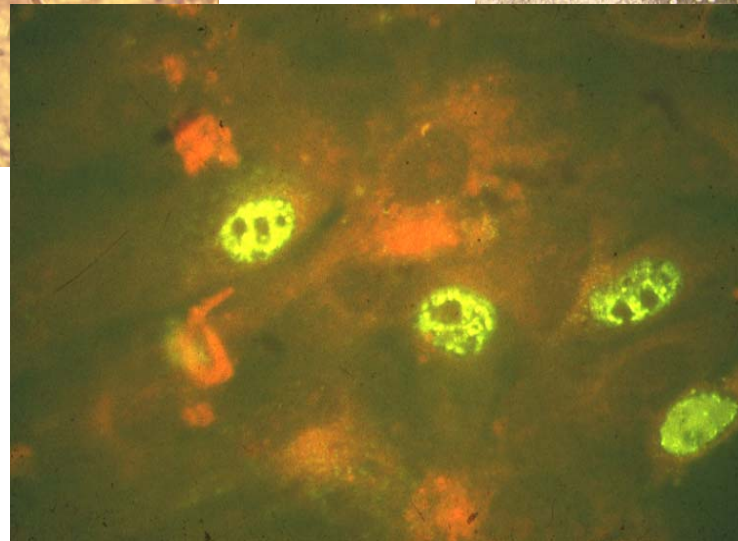
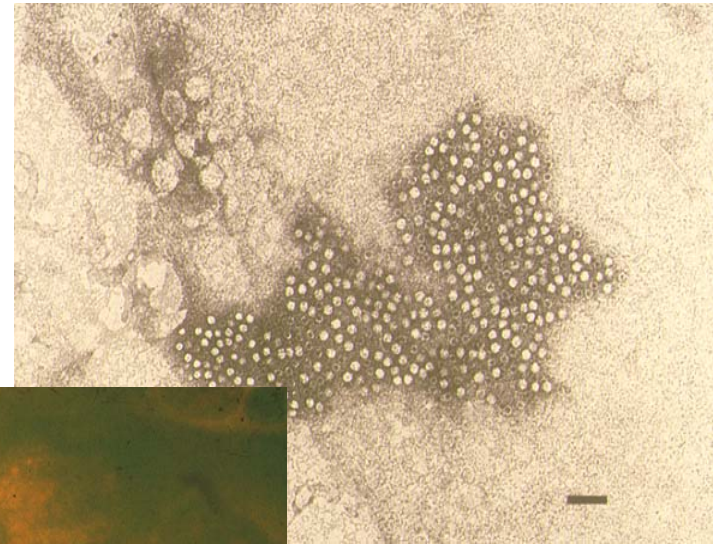
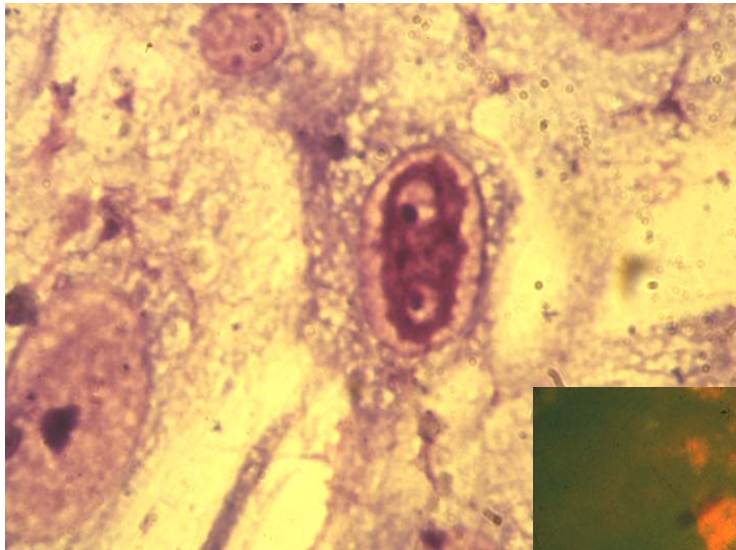


CANINE PARVOVIRUS TYPE 2 (CPV2)



Prof.ssa Annamaria Pratelli

PARVOVIROSI del CANE

MALATTIA INFETTIVA
ALTAMENTE CONTAGIOSA,
CARATTERIZZATA DA
DIARREA EMORRAGICA,
VOMITO INCOERCIBILE E
MORTE DOPO 24-72 h
DA INIZIO PRIMI SINTOMI

CPV

EZIOLOGIA

PARVOVIRIDAE

PARVOVIRINAE

DENSOVIRINAE

GENUS COIPARVOVIRUS

GENUS ERYTHROPARVOVIRUS

GENUS BOCAPARVOVIRUS

GENUS DEPENDOPARVOVIRUS

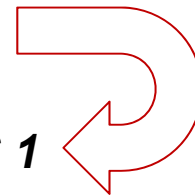
GENUS AMDOPARVOVIRUS

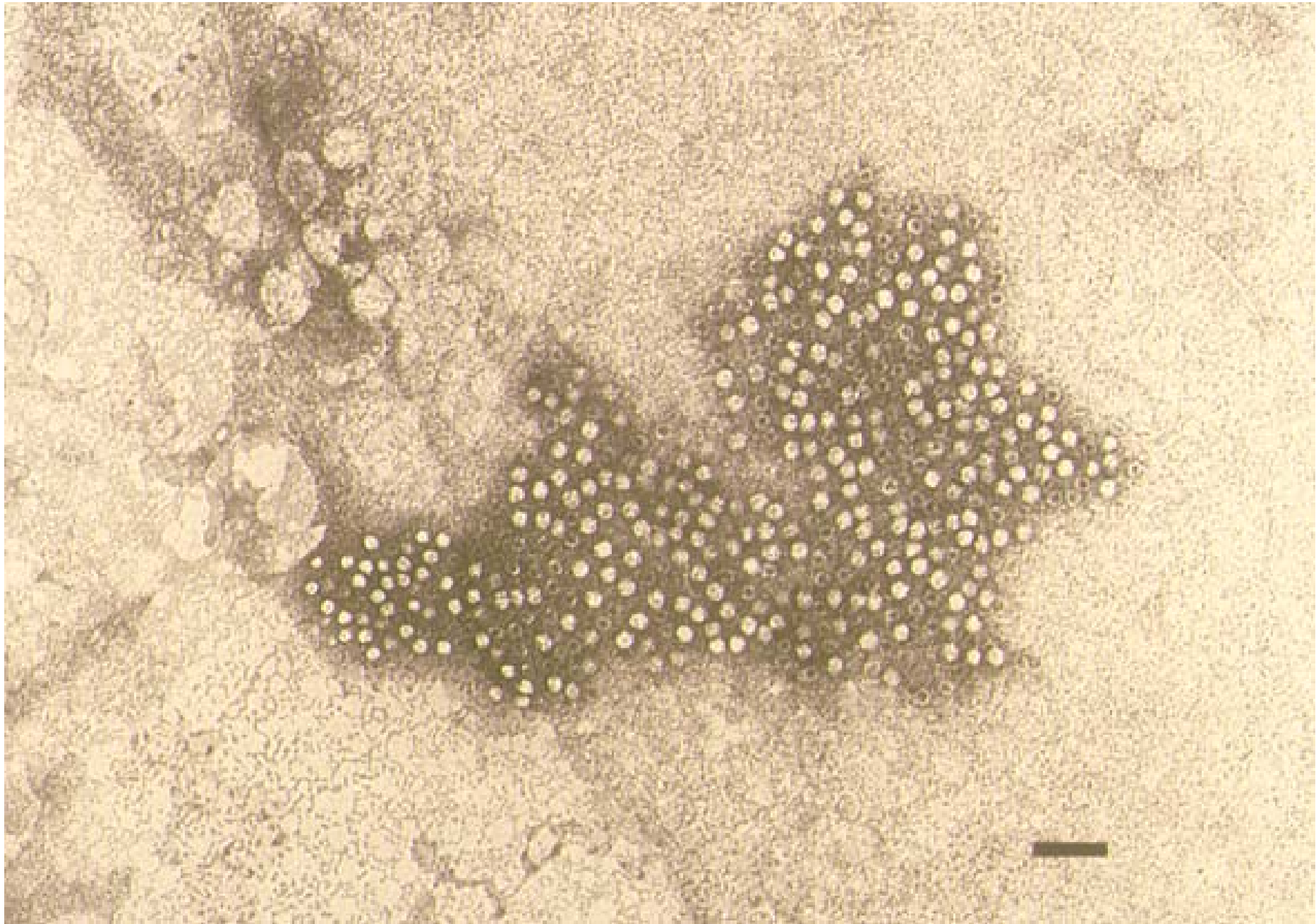
GENUS AVEPARVOVIRUS

GENUS TETRAPARVOVIRUS

GENUS PROTOPARVOVIRUS

CARNIVORE PROTOPARVOVIRUS 1
(CPV-2, FPLV, MPV, BFPV)





CARATTERISTICHE DEI PARVOVIRUS

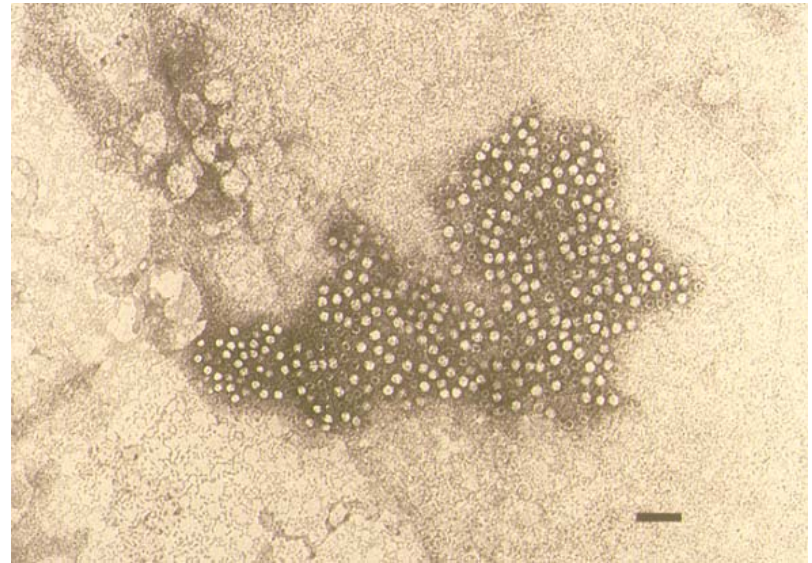
DNA ss (5 kb)

DIAMETRO: 20-30 nm

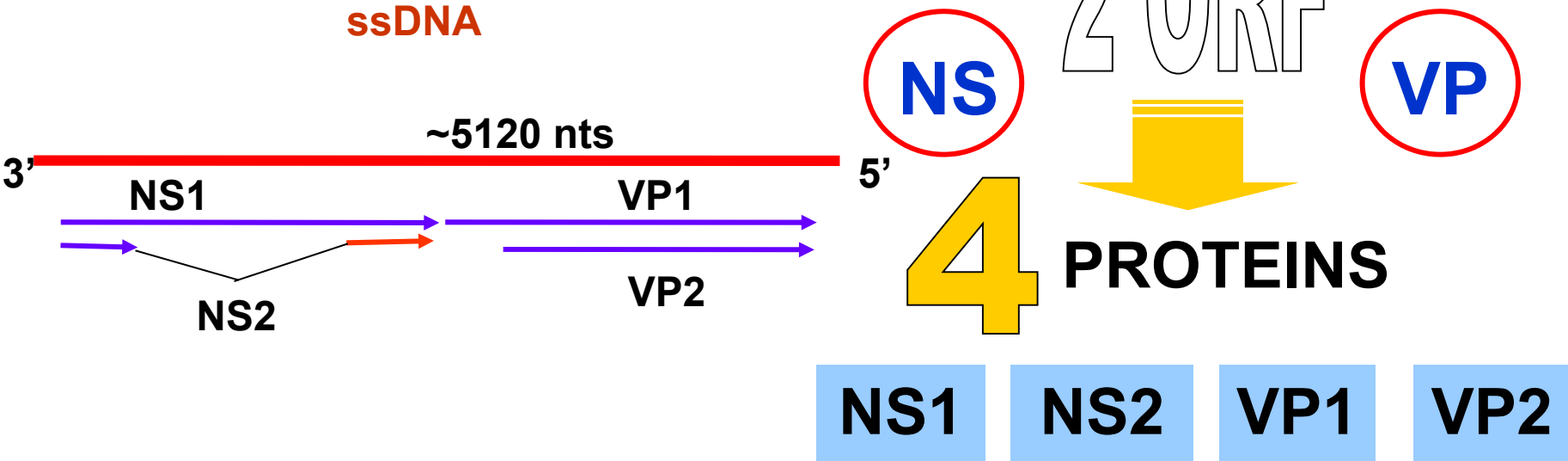
N° CAPSOMERI: 32

FORMA ICOSAEDRICA

NUDO



CPV GENOME



Alternative splicing of the same mRNA

Substitution rates similar to RNA viruses

1.7×10^{-4} substitutions per site per year

HIGH INTRINSEC VARIABILITY (ssDNA)

POSITIVE SELECTION PRESSURE (IMMUNITY)

Schackelton et al., 2005, PNAS

CARATTERISTICHE DEI PARVOVIRUS

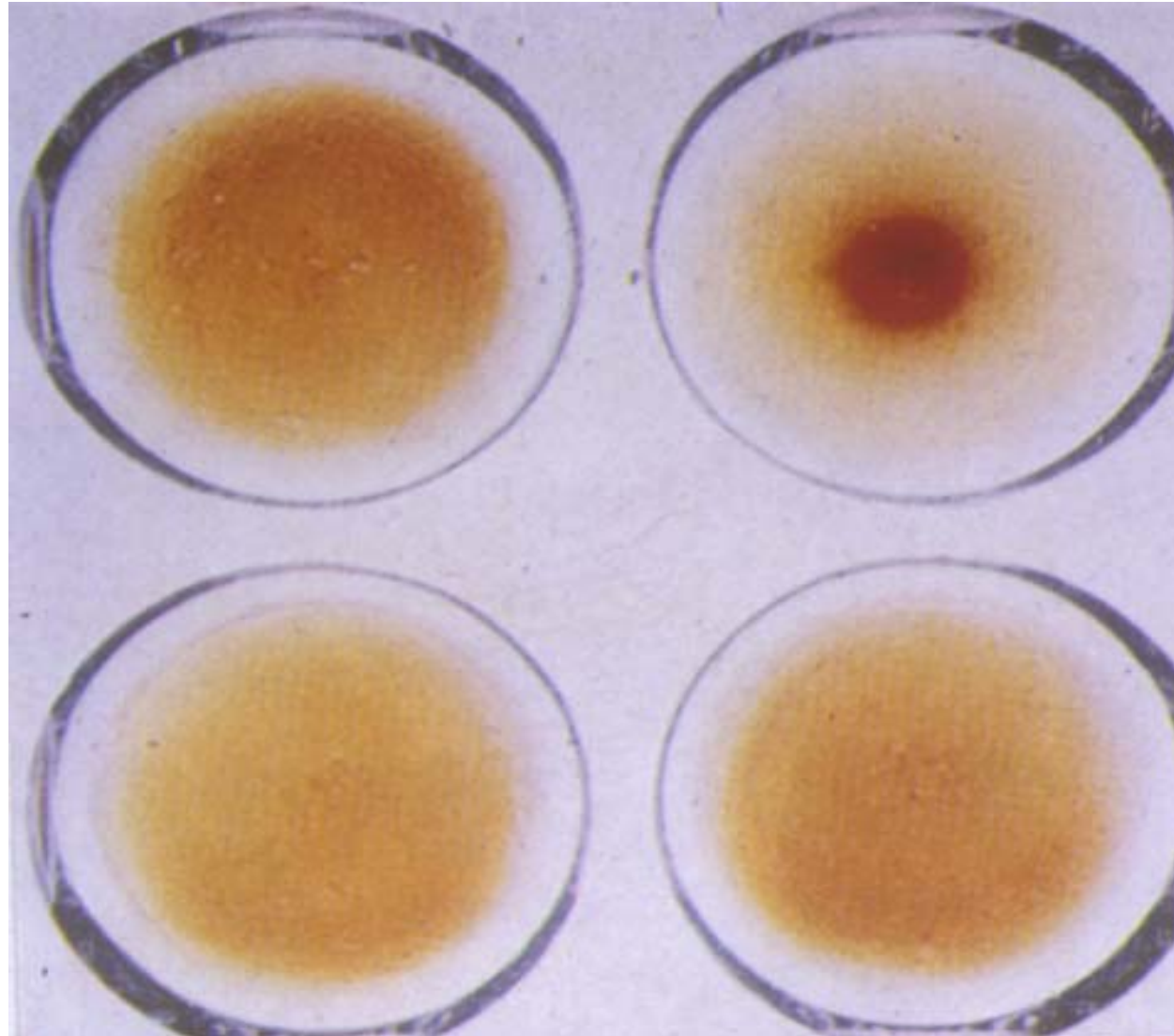
STABILI ALL'AZIONE DELLA
TEMPERATURA (56°C X 1h)

RESISTENTI A VARIAZIONI pH E
AI SOLVENTI DEI LIPIDI

SENSIBILI A SODA, IPOCLORITO,
FORMOLO, β -PROPRIOLATTONE,
IDROSSILAMINA, NaOH, RAGGI UV

CARATTERISTICHE DEI PARVOVIRUS

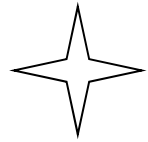
✦ **EMOAGGLUTINANTI**



SPETTRO EMOAGGLUTINANTE

Globuli rossi	Virus		
	CPV	MVC	FPV
SCIMMIA			
SUINO			
CAVALLO			
GATTO			

CARATTERISTICHE DEI PARVOVIRUS



**NON SONO IN GRADO DI
CODIFICARE ALCUN ENZIMA
DI REPLICAZIONE**  **devono
utilizzare enzimi replicativi cellulari
attivi nel nucleo in fase mitotica**

COLTURE CELLULARI

- ❖ **RENE, MILZA E POLMONE DI CANE**
- ❖ **RENE DI GATTO**
- ❖ **CrFK, A-72**

SIEROTIPO UNICO

**CORRELAZIONE ANTIGENICA STRETTA
CON IL VIRUS DELLA
PANLEUCOPENIA FELINA
(FPV)**

STORIA

1970: ISOLAMENTO DA CANI DI UN PARVOVIRUS NON PATOGENO (CPV-1)

1977: TEXAS, PRIMA SEGNALAZIONE DELLA MALATTIA (CPV-2)

1978: LARGA DIFFUSIONE IN USA

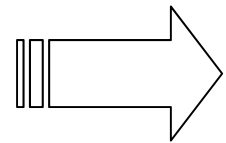
1978: SEGNALAZIONE IN BELGIO

1979: SEGNALAZIONE IN FRANCIA

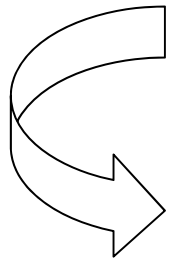
1980: ISOLAMENTO VIRUS IN ITALIA

1978

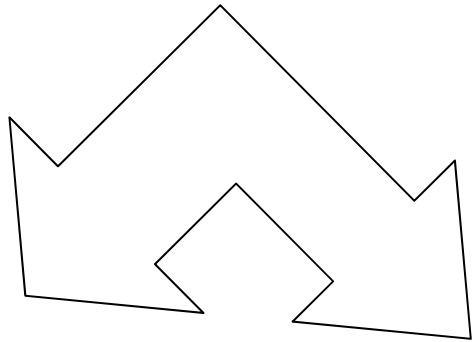
**PARVOVIRUS
DEL CANE**



CPV-2



AGENTE CAUSALE



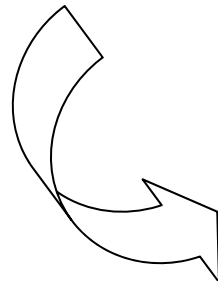
**GASTROENTERITE
EMORRAGICA**

MIOCARDITE



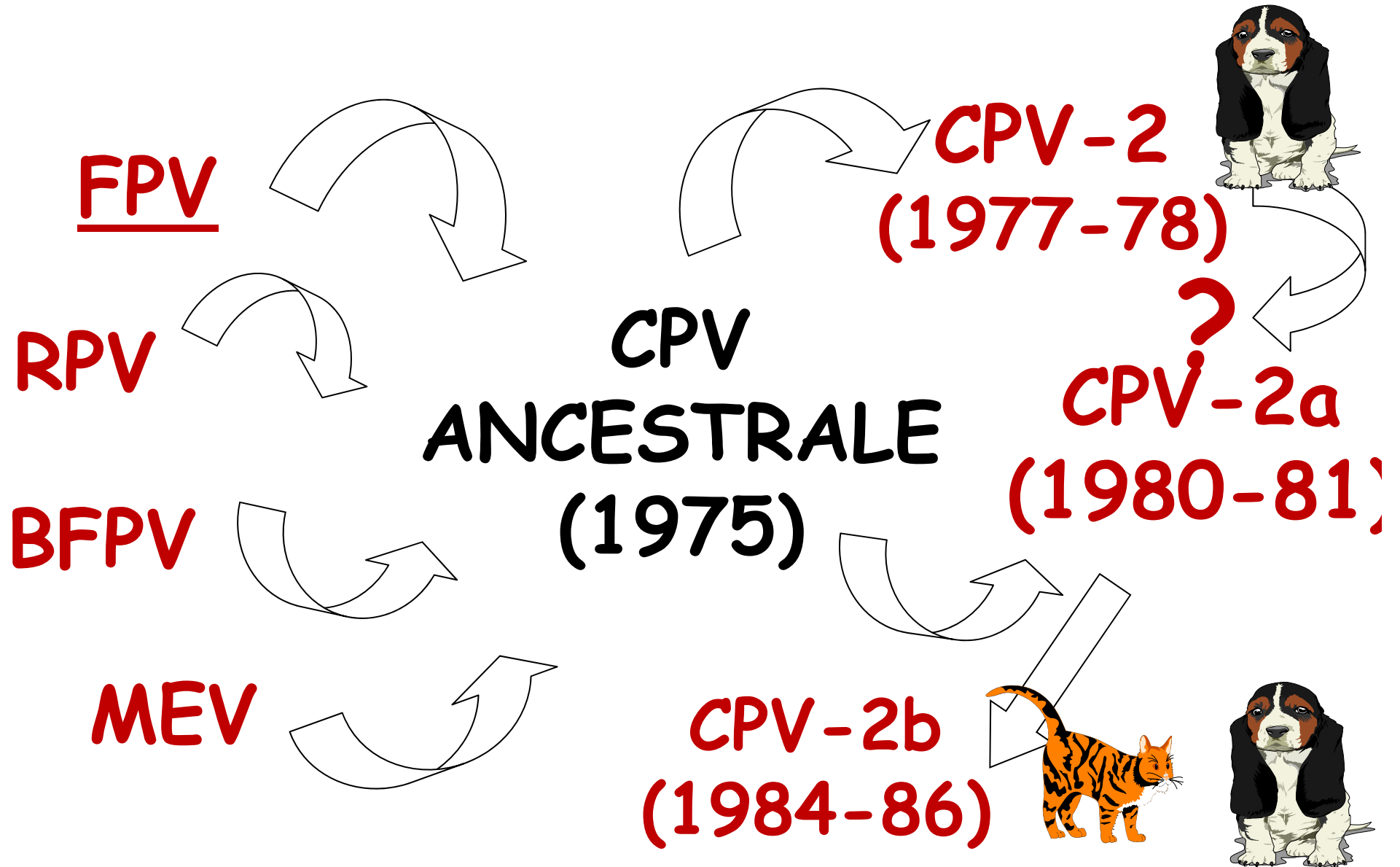
EVOLUZIONE ANTIGENICA CPV-2

- MUTAZIONE DA FPV (in cane o gatto)
- MUTAZIONE FPV VACCINALE
- ORIGINE DA CARNIVORO SELVATICO



VIRUS ANCESTRALE

EVOLUZIONE ANTIGENICA CPV-2



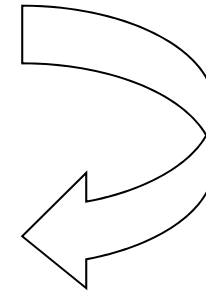
NUOVI SCENARI CPV2

Circolazione virus e prime vaccinazioni: immunità di gregge

CPV2

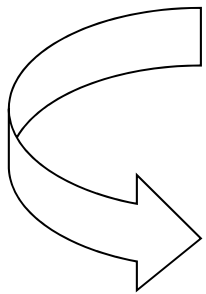
SELEZIONE IMMUNITARIA

CPV2a



SELEZIONE EPITOPO NEUTRALIZZANTE

CPV2b



80 87 101 297 300 305 323 426 555

FPV

Lys Leu

Asn

CPV-2

CPV-2a

Thr

Gly

Tyr

Ile

CPV-2b

Thr

Gly

Tyr

Asp

CPV-2c

Thr

Ala

Gly

Tyr

Glu

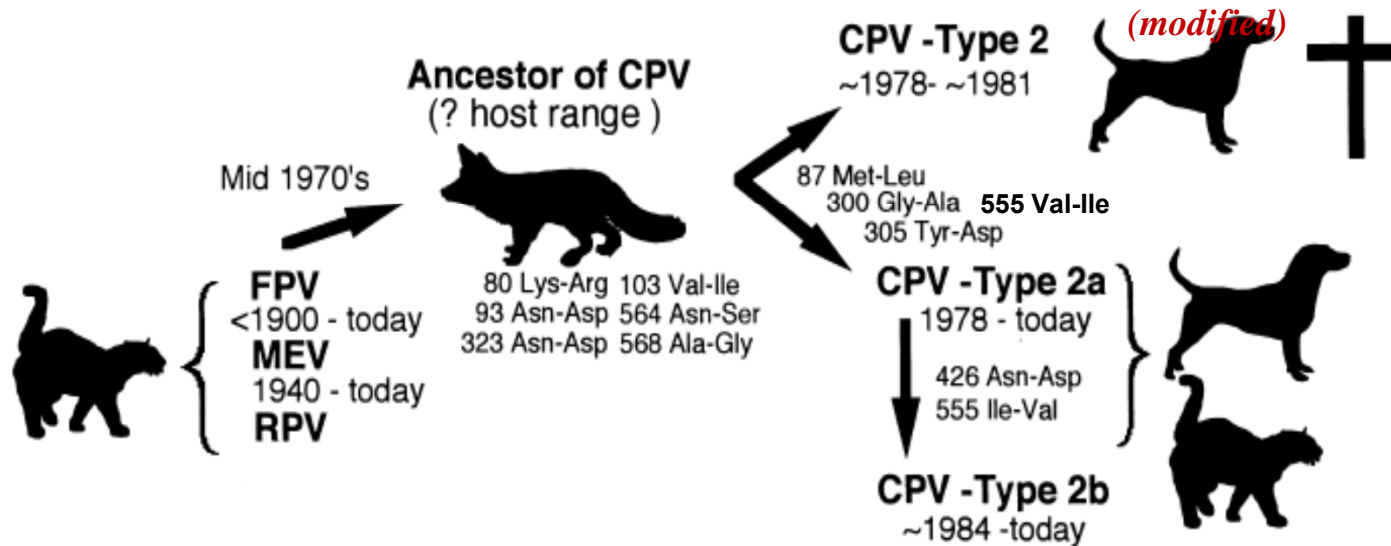
VP2: Sito Ag maggiore

aa 426: epitopo A

Evasione antigenica

EMERGENCE AND EVOLUTION OF CPV-2

U. Truyen/Veterinary Microbiology 69 (1999) 47-50



Variazioni CPV2 e FPV: in antirecettore VP2

Lys93Asn
Asn323Asp

Increased affinity for canine TfR (transferrin receptor)

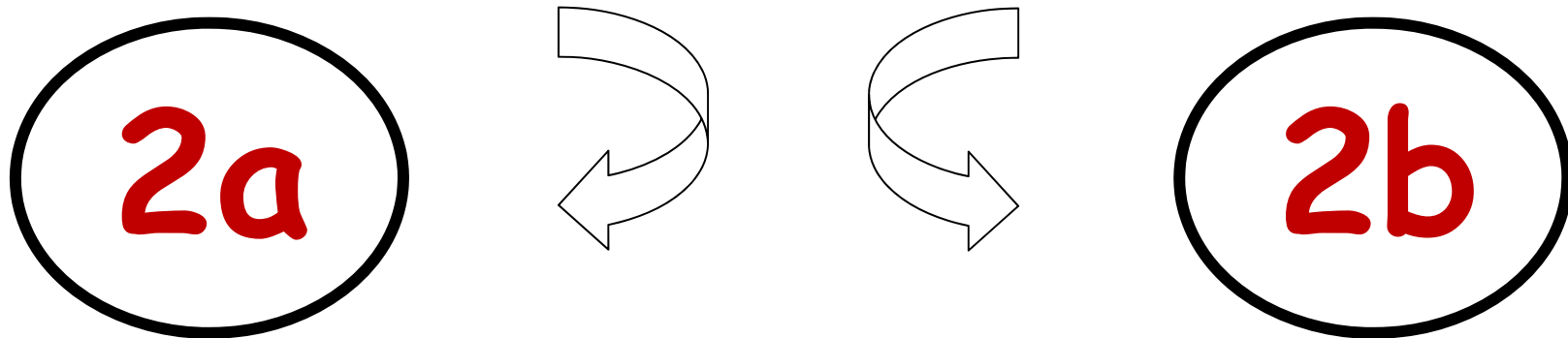
Lys80Arg
Asn564Ser
Ala568Gly

Decreased affinity for feline TfR

Schackelton et al., 2005, PNAS

TIPIZZAZIONE STIPITI CPV-2 con MoAbs (Parrish, 1985)

STIPITI CIRCOLANTI



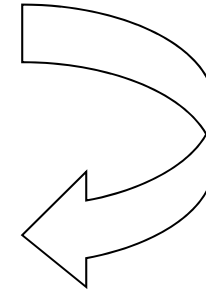
❖ CPV-2 RITROVATO
IN COLONIE ISOLATE DI CANI



NUOVI SCENARI CPV2

CPV2 SELEZIONE IMMUNITARIA

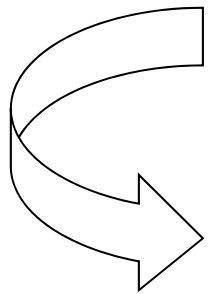
CPV2a



SELEZIONE EPITOPO NEUTRALIZZANTE

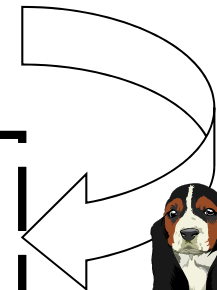
CPV2b

SOSTITUZIONE aa
426 Asp → Glu



(Italia 2001)

CPV2c





Evidence for evolution of canine parvovirus type 2 in Italy

Canio Buonavoglia,¹ Vito Martella,¹ Annamaria Pratelli,¹ Maria Tempesta,¹ Alessandra Cavalli,¹ Domenico Buonavoglia,² Giancarlo Bozzo,¹ Gabriella Elia,¹ Nicola Decaro¹ and Leland Carmichael³

¹Department of Animal Health and Well-being, Faculty of Veterinary Medicine of Bari, S.p. per Casamassima km 3, 70010 Valenzano, Bari, Italy

²Department of Pathology and Infectious Diseases, University of Messina, Messina, Italy

³James Baker Institute, Cornell University, Ithaca, NY, USA

Two isolates of canine parvovirus (CPV) were obtained from dogs affected with severe haemorrhagic diarrhoea. Type 2b antigenic specificity was predicted by both antigenic analysis with monoclonal antibodies and PCR characterization with type-specific primers. Nevertheless, sequence analysis of the capsid protein-encoding gene revealed two amino acid changes. One of the changes affected position 426 (Asp to Glu), in a major antigenic site of the viral capsid, determining the replacement of a residue unique to CPV type 2b. The failure of established typing methods to dis-

Tattersall, 1987), which have low error rates, mutational events affecting the biological properties of parvoviruses presumably occur very rarely. Estimates suggest that the rate of variation is 1×10^{-4} to 4×10^{-4} changes/nt/year (Parrish, 1991; Truyen *et al.*, 1995).

In 1979 and 1980, an antigenic variant of CPV was identified in several different countries by using monoclonal antibodies (MAbs) and the variant was termed CPV type 2a (Parrish *et al.*, 1985, 1991). In the mid 1980s, the virus underwent a further antigenic change, and the new variant was referred to as CPV type 2b (Parrish *et al.*, 1991). Currently, the antigenic variants of CPV have completely replaced the original type 2, and are now widely distributed in various

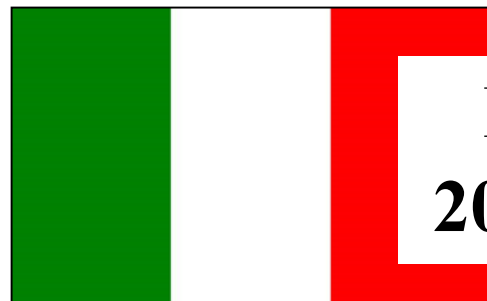
A Canine Parvovirus Mutant Is Spreading in Italy

Vito Martella,^{1*} Alessandra Cavalli,¹ Annamaria Pratelli,¹ Giancarlo Bozzo,¹
Michele Camero,¹ Domenico Buonavoglia,² Donato Narcisi,¹
Maria Tempesta,¹ and Canio Buonavoglia¹

*Department of Animal Health and Wellbeing, University of Bari, Bari,¹ and Department of Pathology
and Infectious Diseases, University of Messina, Messina,² Italy*

Received 13 December 2002/Returned for modification 26 May 2003/Accepted 11 November 2003

By antigenic and genetic characterization of canine parvovirus type 2 (CPV-2) strains collected in 2001 and 2002 in Italy, it was possible to observe the spread of viruses with an unusual mutation, Glu-426, affecting a major antigenic epitope of CPV-2. Out of 67 strains analyzed, 49 (73.13%) were characterized as CPV-2a, 6 (8.95%) were characterized as CPV-2b, and 12 (17.91%) were characterized as the Glu-426 mutant.



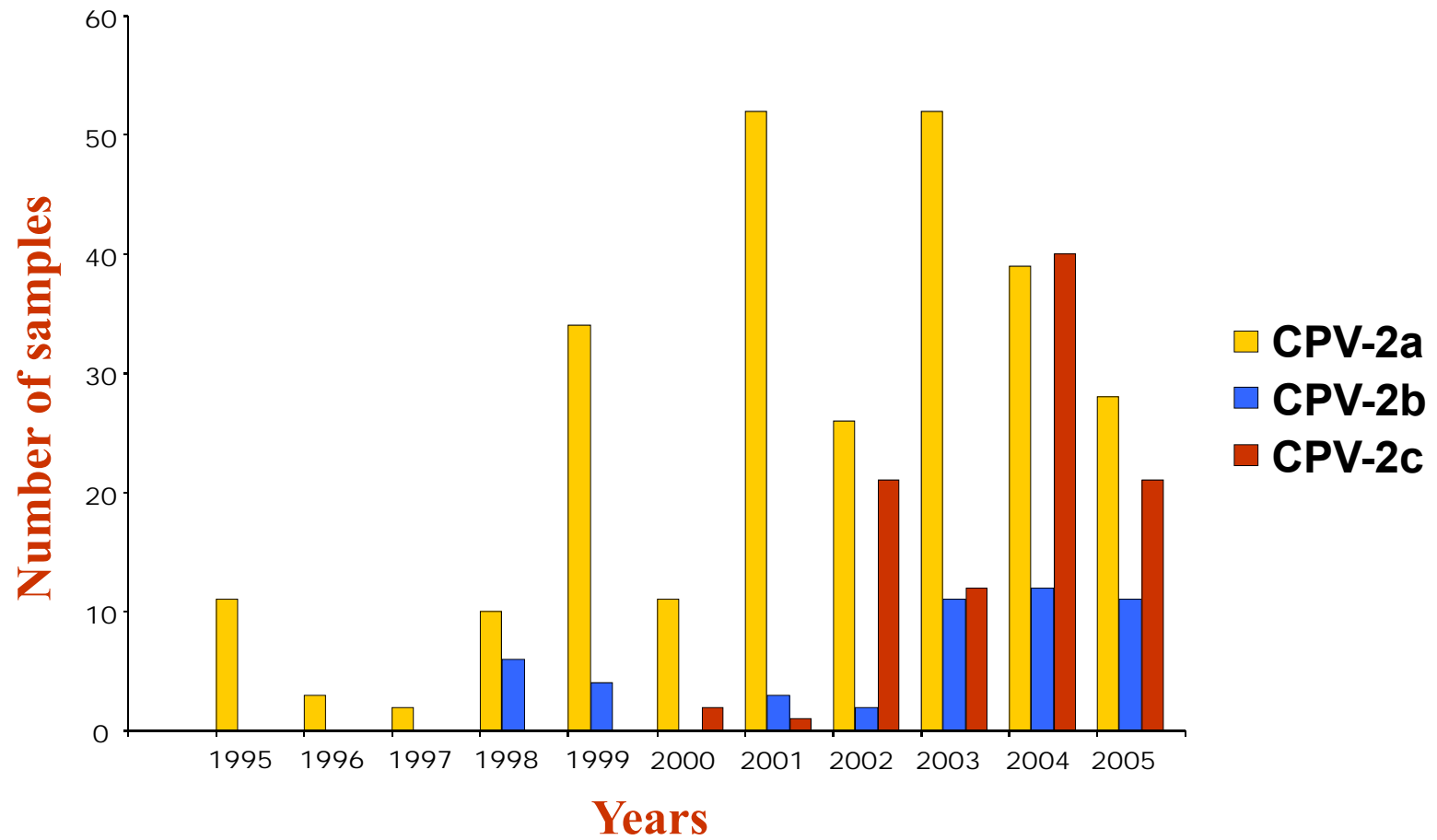
ITALY
2001-2002

CPV-2a	49
CPV-2b	6
CPV-2c	12



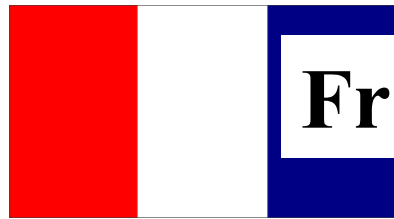
ITALY
1995-2005

CPV-2a	268
CPV-2b	49
CPV-2c	97



TYPE DISTRIBUTION AFTER 2000

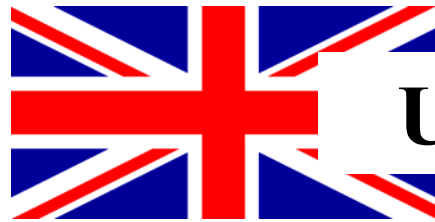
IDENTIFICATION OF CPV-2c IN EUROPEAN COUNTRIES



France, 2005 *Merrial, pers. communic.*



Spain, 2006 *Decaro et al., 2006, J Vet Med B*



UK, 2006 *Diane Addie*



Portugal, 2005-06 *Gertrude Thompson*



Germany, 1996-2006 *Uwe Truyen*

TYPE DISTRIBUTION IN THE WORLD

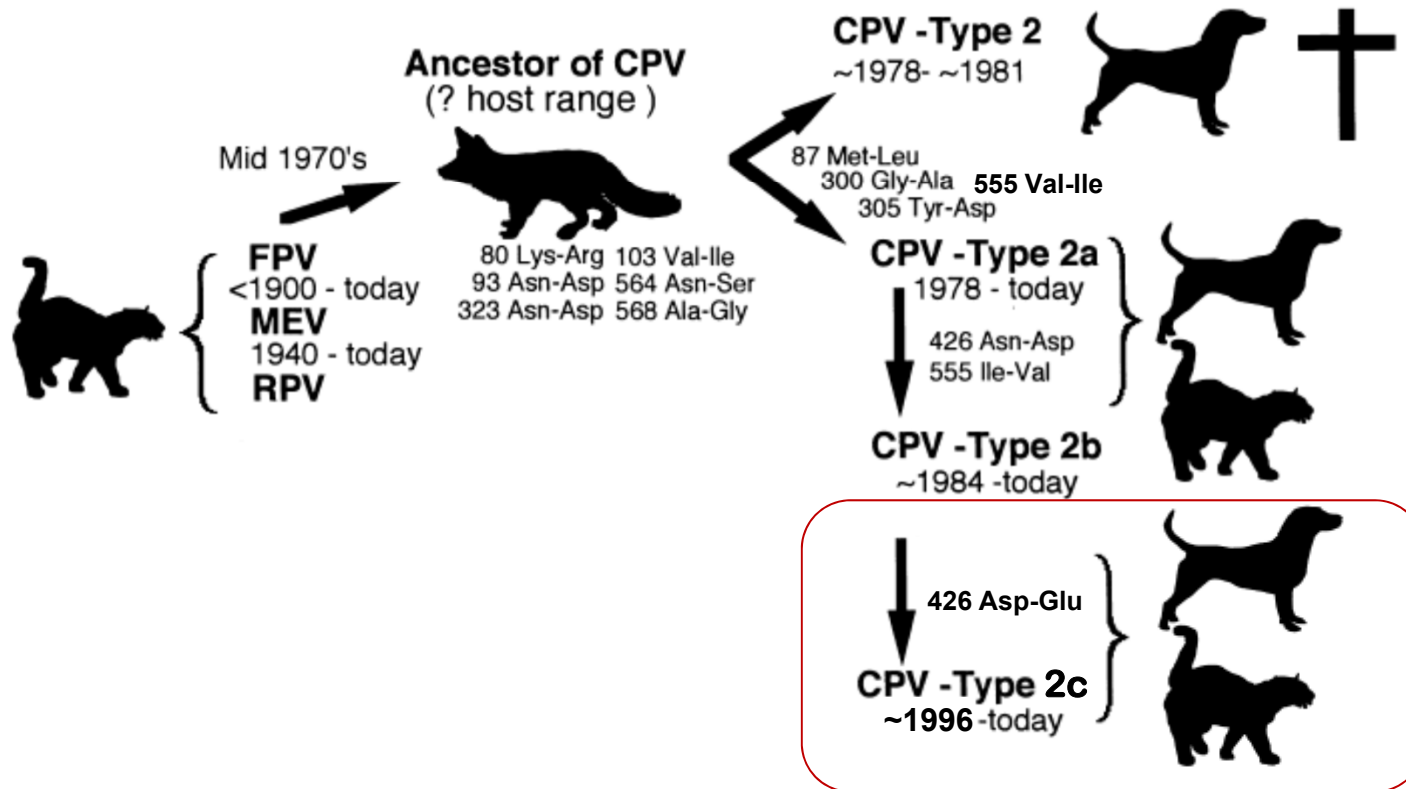
2012

Continent/country	Number of strains detected		
	CPV-2a	CPV-2b	CPV-2c
Europe			
<u>Italy</u>	56	6	62
Portugal	0	16	15
Spain	3	1	9
France	0	9	7
UK	117	182	1
Belgium	17	0	9
Germany	13	18	21
Greece	81	1	2
Switzerland	1	0	0
Czech Republic	1	1	0
Romania	2	0	0
Hungary	27	0	0
Bulgaria	31	9	1
Slovenia	1	0	0
Africa			
Tunisia	15	21	14
North America			
<u>USA</u>	1	36	30
South America			
Uruguay	1	0	24
Argentina	9	4	14
Brazil	37	0	0
Asia			
India	37	4	0
India	^b	^b	3
Taiwan	2	34	0
Korea	119	7	0
Japan	4	21	0
China	27	5	0
Thailand	19	7	0
Oceania			
Australia	41	1	0

Biological features of CPV variants

HOST RANGE

U. Truyen/*Veterinary Microbiology* 69 (1999) 47-50 (modified)



CPV2 infetta cellule di gatto *in vitro*.

Varianti: infettano gatto *in vivo*

RECETTIVITA'

CANE

VOLPE

COYOTE

DINGO

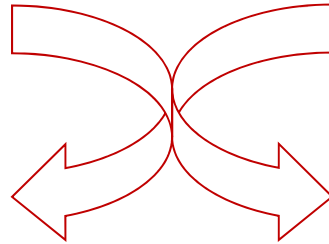
LUPO

PROCIONE

RICERCHE HANNO DIMOSTRATO:
Cani taglia grande più sensibili

DIFFUSIONE

CONTAGIO
DIRETTO



CONTAGIO
INDIRETTO

**MALATTIA PIU' FREQUENTE NEL
PERIODO COMPRESO TRA
OTTOBRE ED APRILE**

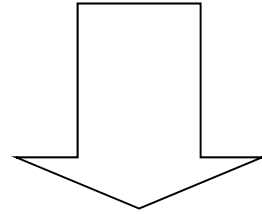
✓ L'ANIMALE INFETTO ELIMINA IL VIRUS
ATTRAVERSO GLI ESCRETI ED I SECRETI
IN GRANDE QUANTITA' PRIMA DELLA
COMPARSA DEI SINTOMI CLINICI

✓ VIRUS NOTEVOLMENTE RESISTENTE
NELL'AMBIENTE

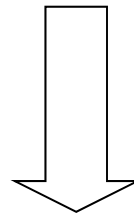
✓ DIMOSTRATO IL PASSAGGIO
PER VIA TRANSPLACENTARE

PATOGENESI

PENETRAZIONE

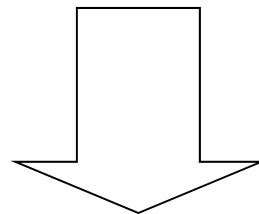


**LINFONODI FARINGEI
PLACCHE DEL PEYER**

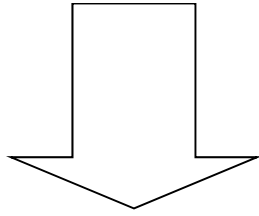


**Viremia linfocito associata
1-3 gg**

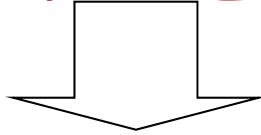
**DISTRETTI LINFATICI
(milza, timo, linfonodi)**



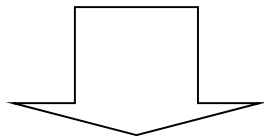
4-5 gg



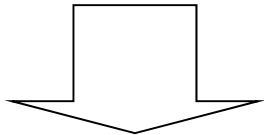
INT. TENUE



**EPITELIO GERMINALE di
CRIPTE del DUODENO
VILLI del DIGIUNO e ILEO**

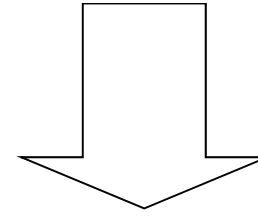


LISI (Alterazione normale
turn-over epitelio)

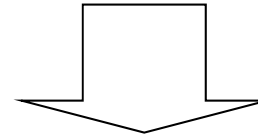


DIARREA

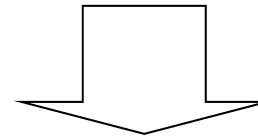
Escrezione: fino a 40-50 gg!!!!



MIOCARDIO



**FIBRILLAZIONI
EXTRASISTOLE**



MORTE

**BIOLOGICAL DIFFERENCES BETWEEN
CPV-2 AND ITS ANTIGENIC VARIANTS**

CPV-2a CPV-2b CPV-2c

INCREASED PATHOGENICITY

- **INCUBATION PERIOD < 4-5 d**
- **MORE SEVERE ENTERITIS**
- **> MORTALITY RATES**
- **> HA TITRES IN THE FAECES**
- **< INFECTIOUS TITRES/GR FAECES**

SINTOMATOLOGIA

PERIODO D'INCUBAZIONE: 4-5 gg ...

❖ **FORMA CARDIACA**

❖ **FORMA DIGESTIVA**

❖ **FORMA SUBCLINICA**

FORMA SUBCLINICA

CARATTERIZZATA SOLO DA
DEPRESSIONE
E/O ANORESSIA
LEUCOPENIA

Problema nei canili e negli allevamenti!!

FORMA CARDIACA

Sporadica!

✓ **MORTALE PER I CUCCIOLI
DI 2-4 SETTIMANE**

✓ **CARATTERIZZATA DA
MIOCARDITE ACUTA**

- A volte preceduta da sintomi gastroenterici
(dispnea, vomito, lamenti)

- A volte assenza sintomi: ECG

Predisporre patologie degenerative miocardio

FORMA DIGESTIVA

CLINICAL SIGNS OF PARVOVIROSIS

- HAEMORRHAGIC DIARRHOEA
- VOMITING
- RAPID DEHYDRATION
- FEVER (inconstant)
- LEUKOPOENIA
(<3000 WBC/mm³)
- MORTALITY

Clinical course depends on
Ab titres at the moment of infection

LESIONI ANATOMO-ISTOPATOLOGICHE

ENTERITE EMORRAGICA

 Intestino con materiale emorragico o vuoto

 PICCOLE ULCERE SULLA MUCOSA GASTRICA

 AUMENTO VOLUME LINFONODI e PLACCHE PEYER
Emorragie su corticale

 DEPLEZIONE E NECROSI DEI TESSUTI LINFOIDI

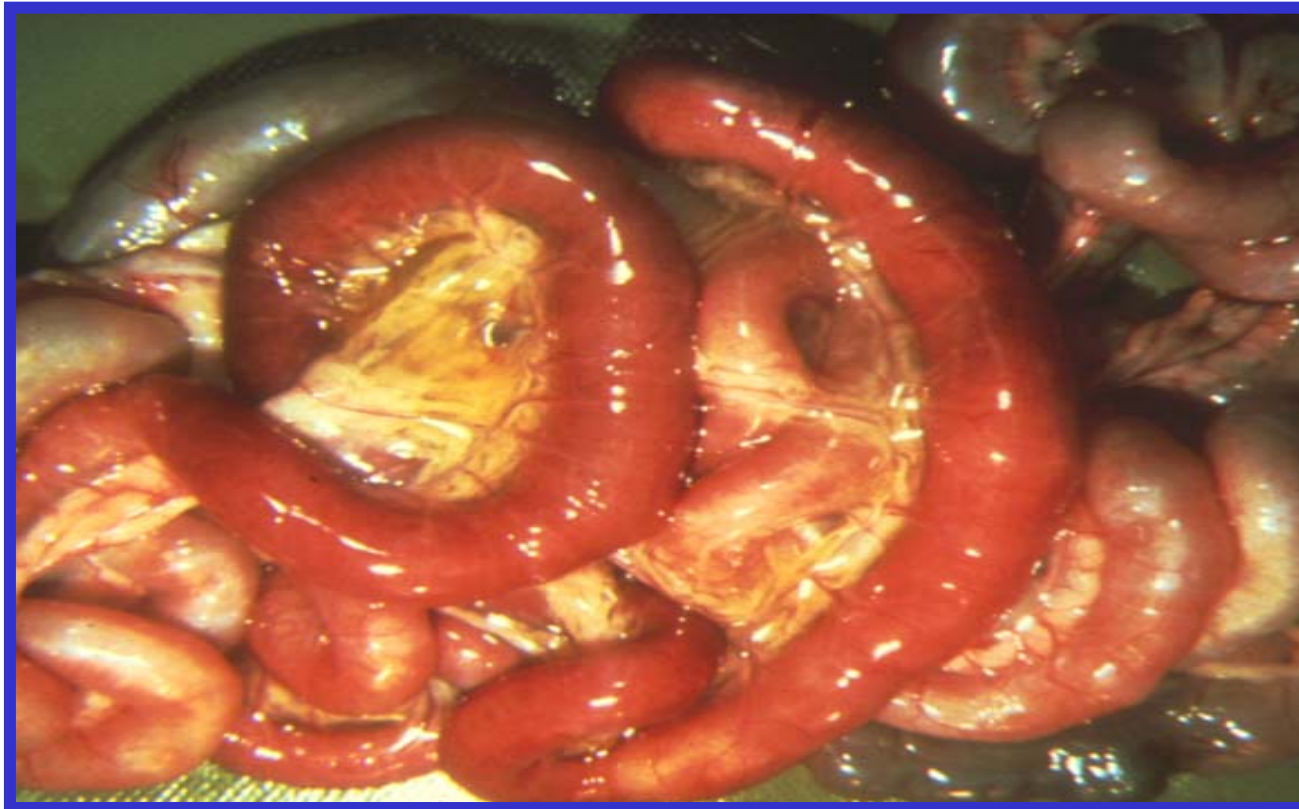
 EROSIONE VILLI INTESTINALI E LORO FUSIONE

 NECROSI MULTIFOCALE delle CRIPTE

 INCLUSIONI NUCLEARI DI TIPO A DI COWDRY

 Edema polmonare e alveolite

INFIAMMAZIONE EMORRAGICA DELLA MUCOSA INTESTINALE



DEGENERAZIONE DELLE FIBRE MIOCARDICHE



TALVOLTA PRESENZA DI FIBROSI MIOCARDICA

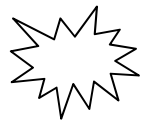
Edema polmonare

Pareti flaccide e aree di necrosi

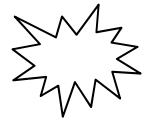
**Istologia: miocardite non suppurativa
e inclusi**

DIAGNOSI

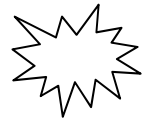
DIAGNOSI CLINICA



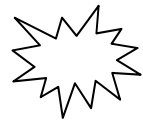
VOMITO



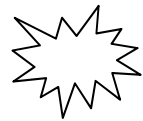
DIARREA EMORRAGICA



ODORE NAUSEABONDO



RAPIDA DISIDRATAZIONE



**REPERTI EMATOLOGICI
(< 3000 gl. bianchi/mm³)**

DIAGNOSI DIFFERENZIALE

TURBE ENTERICHE LEGATE A SVEZZAMENTO:
diarrea mai emorragica, manca la febbre

AVVELENAMENTI DA ANTICOAGULANTI:
sensibili alla somministrazione di vit. K
e di antidoti specifici

INFESTAZIONI DA ACARI:
esame delle feci risolutivo

INFEZIONI BATTERICHE:
sensibili a chemioterapici

INFEZIONI VIRALI:

CIMURRO

SINTOMI ENTERICI POCO EVIDENTI RISPETTO A RESPIRATORI

SONO OSSERVABILI SINTOMI NERVOSI
E LESIONI ALLA CUTE E CUSCINETTI PLANTARI

EPATITE INFETTIVA

DIARREA RARAMENTE EMORRAGICA

PRESENTI ITTERO, SUB-ITTERO

SINTOMI NERVOSI E LESIONI CORNEALI

CORONAVIRUS E ROTAVIRUS

✓ DIARREA CON TRACCE DI SANGUE

✓ IN GENERE DECORSO BENIGNO

MORTE PER COMPLICAZIONI BATTERICHE SECONDARIE

DIAGNOSI DI LABORATORIO

DIAGNOSI VIROLOGICA

- ESAME DELLE FECI AL M.E.
- ATTIVITA' EA DEL VIRUS NELLE FECI
- IF DIRETTA ED INDIRETTA
- ISOLAMENTO IN COLTURE DI TESSUTO
- DIAGNOSI BIOMOLECOLARE

DIAGNOSI SIEROLOGICA

- REAZIONE DI IEA
- REAZIONE DI SN

TRADITIONAL METHODS

CPV DETECTION

ICT



Are ELISA-based assays able to detect efficiently CPV-2c?

Rates of test failures increased in the last years paralleling the increased frequency of CPV-2c detection

Kapil et al., 2007, J Clin Microbiol

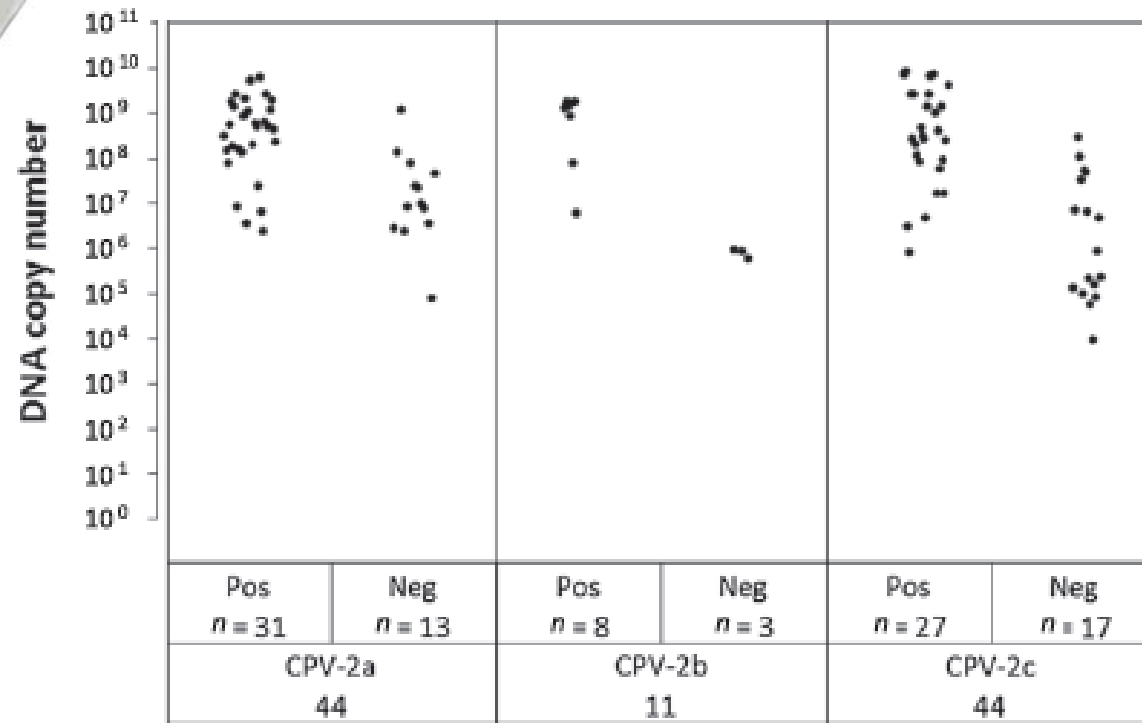
CPV DETECTION

The Veterinary Journal xxx (2013) xxx-xxx

Evaluation of an in-clinic assay for the diagnosis of canine parvovirus



CPV-2a, 44
CPV-2b, 11
CPV-2c, 44

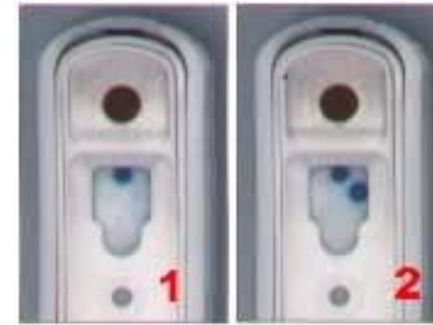


**CPV-2c was detected with the same efficiency
as the other antigenic variants!**

CPV DETECTION

TRADITIONAL METHODS

ICT



Poorly sensitive in the late stage of infection



Low viral titers

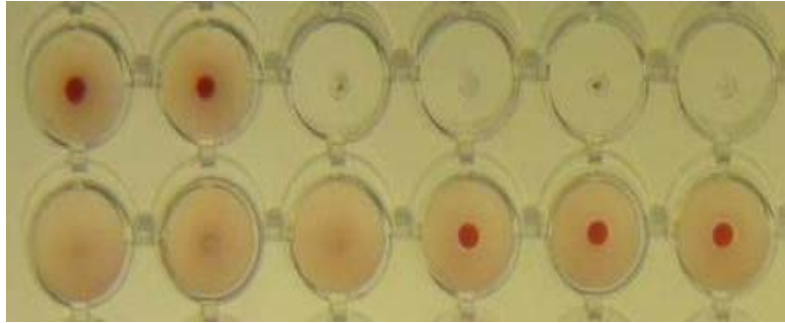


Abs in the gut lumen

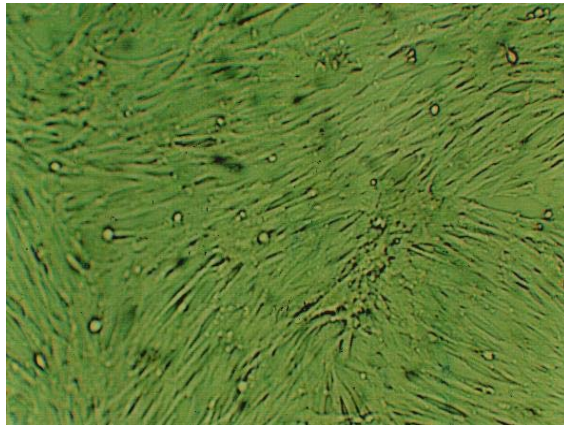
TRADITIONAL METHODS

CPV DETECTION

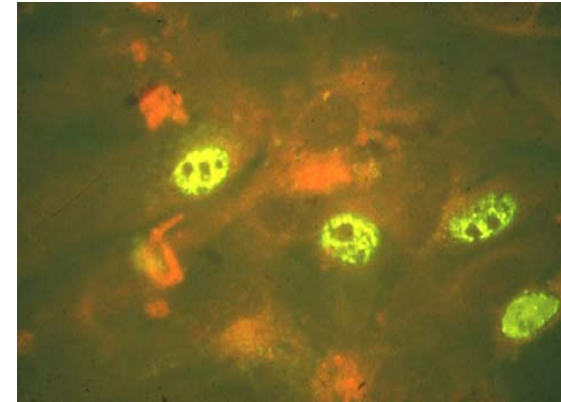
HA



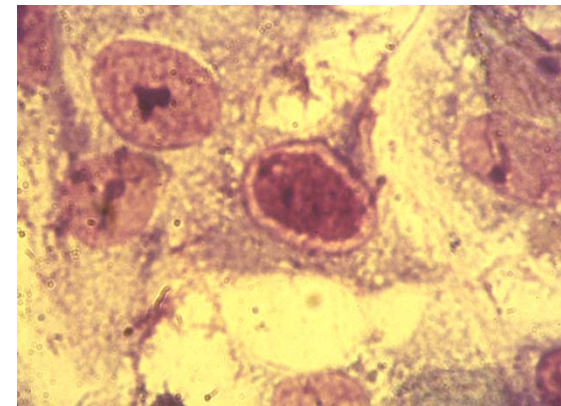
VI



IFA



H/E



Poorly sensitive in the late stage of infection

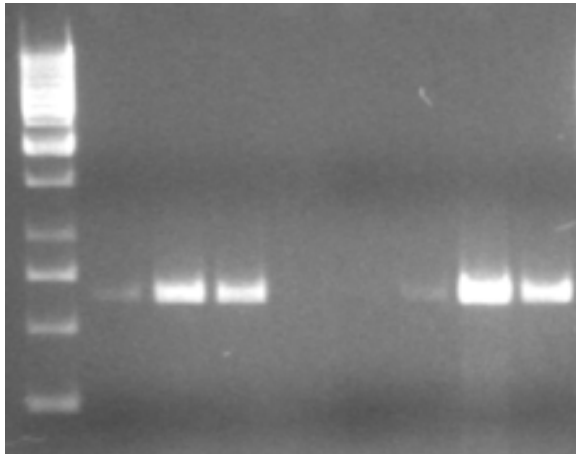
Low viral titres

Abs in the gut lumen

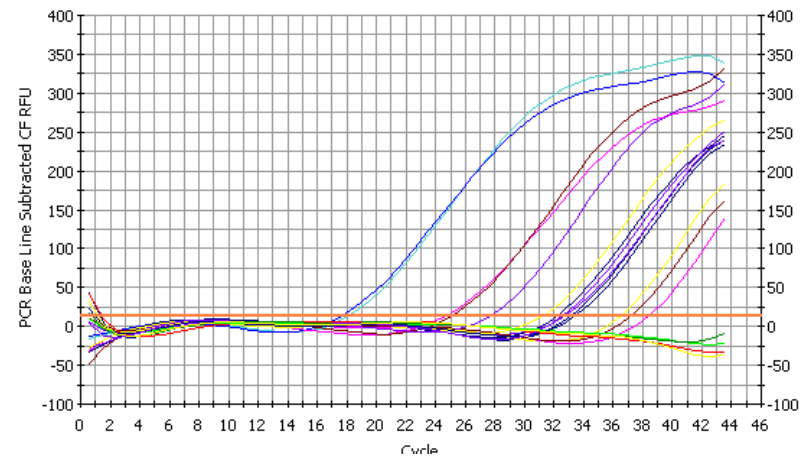
CPV DETECTION

INNOVATIVE METHODS

PCR



REAL-TIME PCR WITH TAQMAN PROBES



Highly sensitive



**Detection of
nucleic acid**

CPV CHARACTERISATION

TRADITIONAL METHODS

✱ HI using MoAbs ----->

A4E3, B4A2,
C1D1, B4E1

C. Parrish

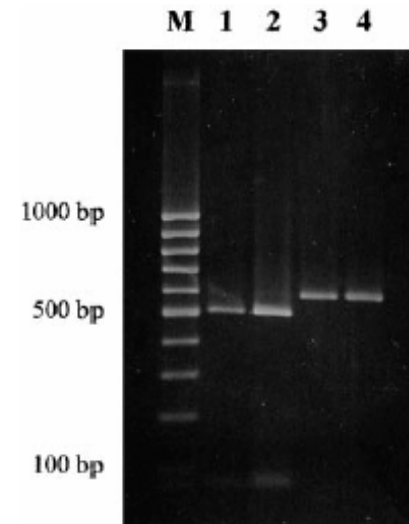
21C3

M.

Nakamura

✱ PCR-RFLP with MbolI

Time consuming
and
often inconclusive



✱ SEQUENCE ANALYSIS **VP2**

CPV CHARACTERISATION

INNOVATIVE METHODS

**REAL-TIME PCR
WITH MGB PROBES**

**DISCRIMINATION BETWEEN
VACCINE AND FIELD STRAINS**

Test 2/v

Test D/f Test 39/f

Diagnostic dilemma

**Positivity to CPV tests
of dogs with post-vaccinal
gastroenteritis**

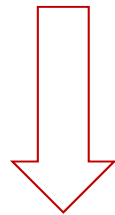
**Solution of legal issues between
owners, breeders, vets, companies**

PROFILASSI

CPV2 eliminato per 7-10 gg

DIRETTA

INDIRETTA



- Isolamento cuccioli infetti
 - Disinfezioni

STORIA

CPV2

1978

INATTIVATI

VACCINI ETEROLOGHI (FPV)



DOSE VACCINO > 1000 VOLTE
RISPETTO A GATTO

1982

VACCINI OMOLOGHI



VACCINI OMOLOGHI SPENTI

★ SCARSA IMMUNOGENICITA'

★ BREVE PROTEZIONE

DOPO 3 MESI DA VACCINAZIONE TITOLI 1:40

★ INFEZIONI SUBCLINICHE
DOPO 2 SETTIMANE

★ ELIMINAZIONE VIRUS IN FECE



VACCINI OMOLOGHI VIVI

★ STABILI

★ RAPIDA SIEROCONVERSIONE

★ TITOLI HI > 1:320 PER
OLTRE 5 ANNI!!!



CPV vaccination

VACCINATION

✓ Crucial in pups

Problem:

MDA INTERFERENCE

MDA titres in pups are related to the antibody titres in dams

MLV VACCINES

CPV-2

CPV-2b



FREQUENTI EPISODI DI MALATTIA POST VACCINAZIONE

- ✦ NO x REVERSIONE PATOGENICITA'
- ✦ NO x SCARSA EFFICACIA

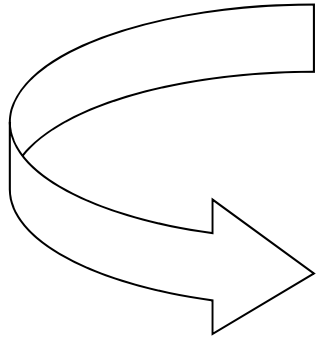
INTERFERENZA MDA

NO IMMUNIZZAZIONE

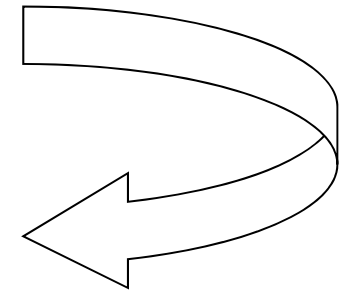


ANTICORPI MATERNI

TRASMISSIONE



**VIA
PLACENTARE**
(uomo, roditori)



**VIA
COLOSTRALE**
(ruminanti, suini)

CARNIVORI

PREALLATTAMENTO
5-10%

POSTALLATTAMENTO
70-80%

FUNZIONI MDA

☀️ PROTEZIONE PASSIVA NEONATI

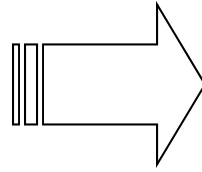
☀️ INTERFERENZA IMMUNIZZAZIONE ATTIVA

TITOLO MDA

**DIRETTAMENTE PROPORZIONALE
TITOLO MADRE**



MDA



COLOSTRO



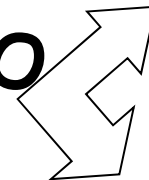
ASSORBIMENTO ENTRO 12-24h

MDA

EMIVITA 8,5 - 9,5 gg

GAP IMMUNOLOGICO

IN PARTICOLARE MOMENTO



NON PROTEGGONO

**INTERFERISCONO
CON VACCINO**

