

Morbillivirus e Cetacei: Una Storia in Continua Evoluzione

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Marine”, Museo di Storia Naturale dell’Università
di Pisa, Calci (Pisa), 30. Ottobre. 2017*



UNIVERSITÀ
DEGLI STUDI
DI TERAMO

Morbillivirus (CeMV)

Toxoplasma gondii

Brucella ceti

Herpesvirus

West Nile virus.....????

Contesti e «scenari» ecologici ed epidemiologici di riferimento

Cambiamenti climatici (Global warming)

2016 = anno più caldo finora registrato
(*Scientific American*, 2017)

Emerging Infectious Diseases (EIDs)
Infezioni trasmesse da artropodi - Potenziale zoonosico delle
EIDs (!!!)

Harmful Algal Blooms (*PNAS*, 2017)

Toward 10 Billion

New projections by the United Nations suggest the world's population may surpass 10 billion by 2100 — with Asia and Africa far and away the most populous regions.

6 billion people

**WORLD
POPULATION**

4

2

0

10

8

**MIDRANGE
PROJECTIONS**

2100, IN
BILLIONS

Oceania
0.07

North
America
0.53

Europe
0.67

Latin
America,
Carib-
bean
0.69

Africa
3.57

Asia
4.60

1960 1980 2000 2020 2040 2060 2080 2100

«More plastic than fish in the sea by 2050»
(*World Economic Forum Report, 2016*)



Microplastiche ed organismi invertebrati (microorganismi?)
(*Carlton et al., Science, 2017; Di Guardo & Mazzariol, Science, 2017*)

Still within 2050, 15-40% of all the species
living in our Planet could become extinct
(*Thomas et al., Nature, 2013*)

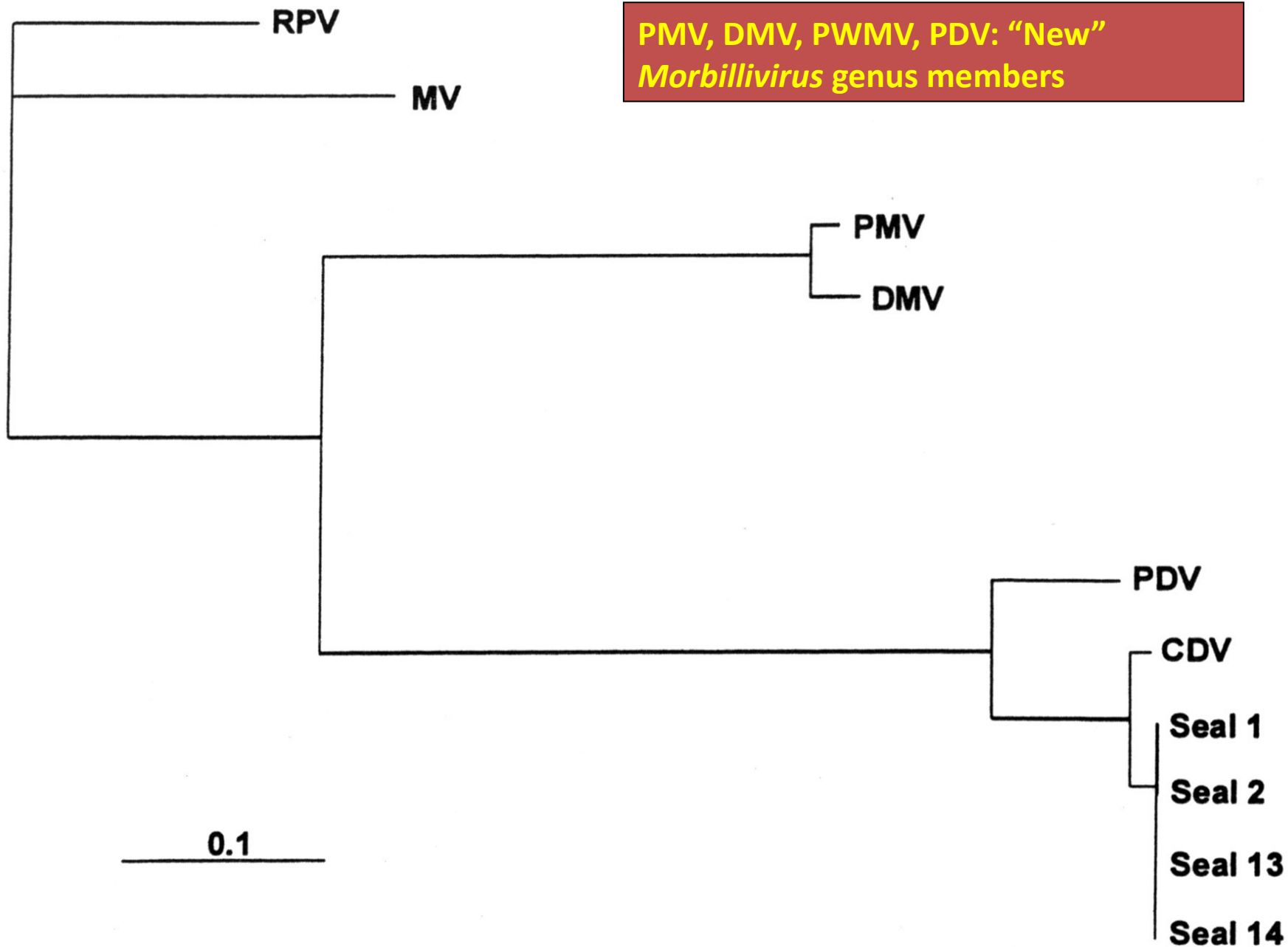
PARAMYXOVIRIDAE

***Morbillivirus* genus**

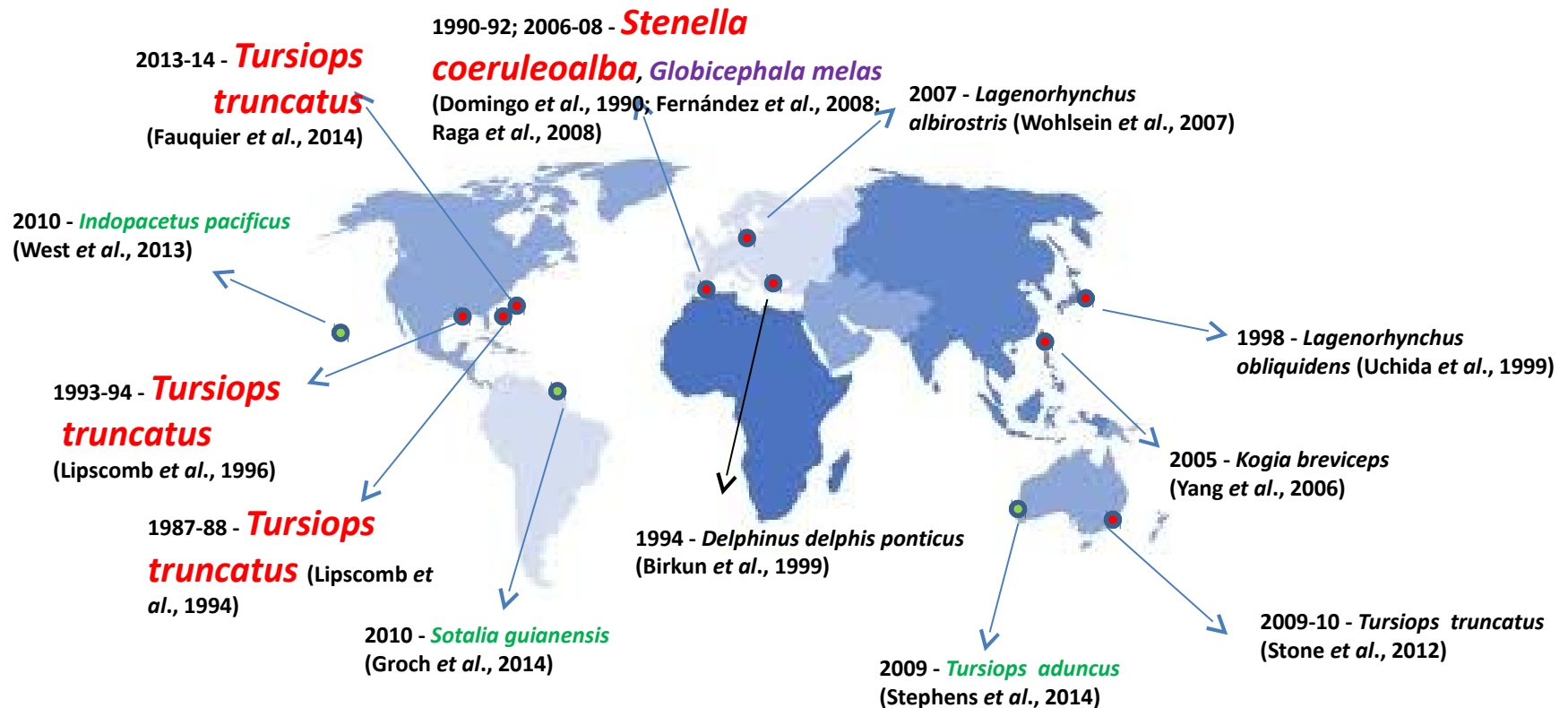
- Large RNA-viruses (structural vs non-structural antigens)
- Lacking neuraminidase
- Serologically related
- Highly pathogenic



**PMV, DMV, PWMV, PDV: "New"
Morbillivirus genus members**



CeMV and DMV Epidemics and Infections Worldwide

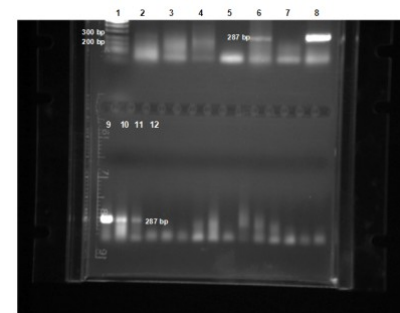
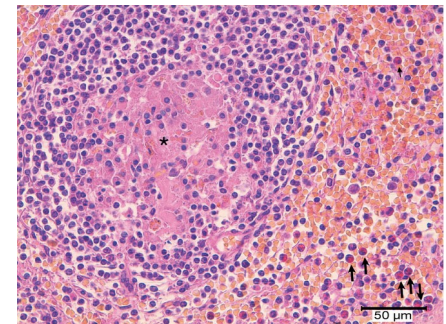
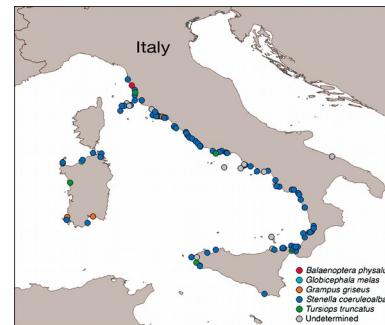


DMV Epidemics in the Western Mediterranean

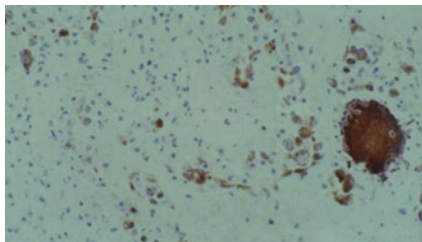
1. 1990-'92 (Domingo *et al.*, 1990, 1992; Di Guardo *et al.*, 1992, 1995)
2. 2006-'08 (Fernández *et al.*, 2008; Raga *et al.*, 2008; Keck *et al.*, 2010)
3. 2011 (Rubio-Guerri *et al.*, 2013)
4. 2013 (Casalone *et al.*, 2014)



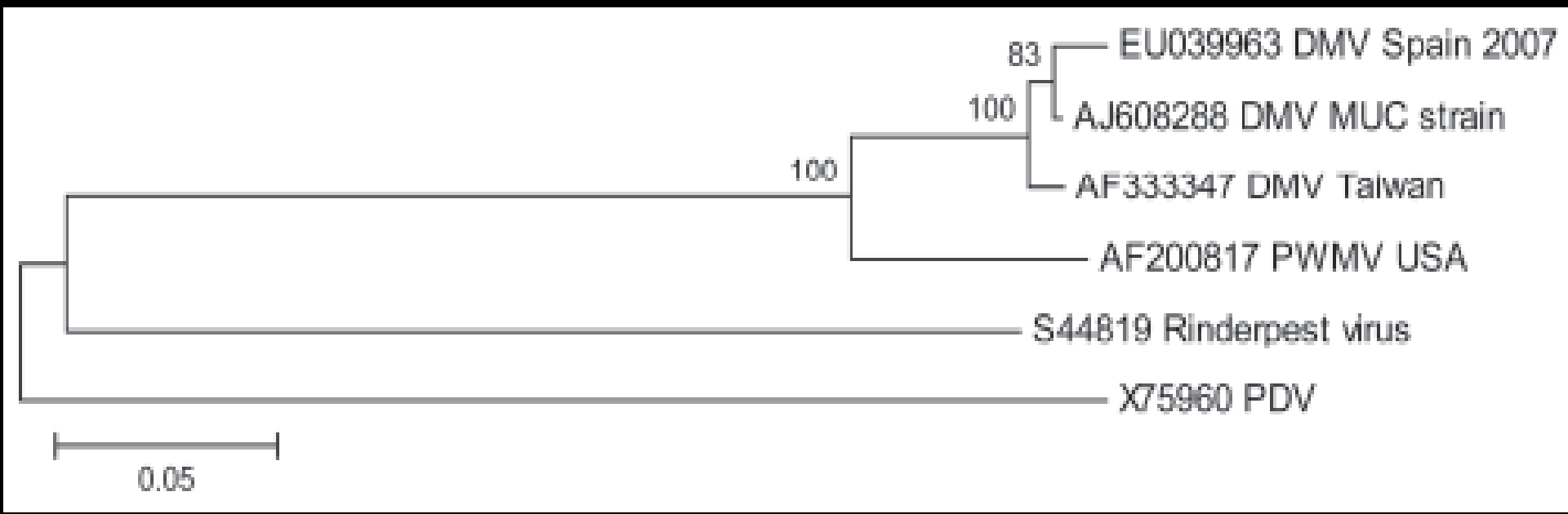
- 122 cetaceans (mainly striped dolphins) found stranded (January-March 2013)
- 24/57 (42%) molecularly (RT-PCR) positive for DMV



Lane 1: DNA Ladder
 Lanes 2-5: campioni di linfonodi e cervello negativi provenienti da due esemplari di Stenella
 Lane 6: campione di cervello positivo proveniente da un esemplare di Tursiops
 Lane 7: campione di polmone negativo proveniente da un esemplare di Tursiops
 Lanes 8 e 9: controllo positivo (CDV Onderstepoort strain)
 Lane 10: campione di rene positivo proveniente da un esemplare di Tursiops
 Lane 11: campione di linfonodi positivo proveniente da un esemplare di Balenoptera



Di Guardo *et al.*, 1992

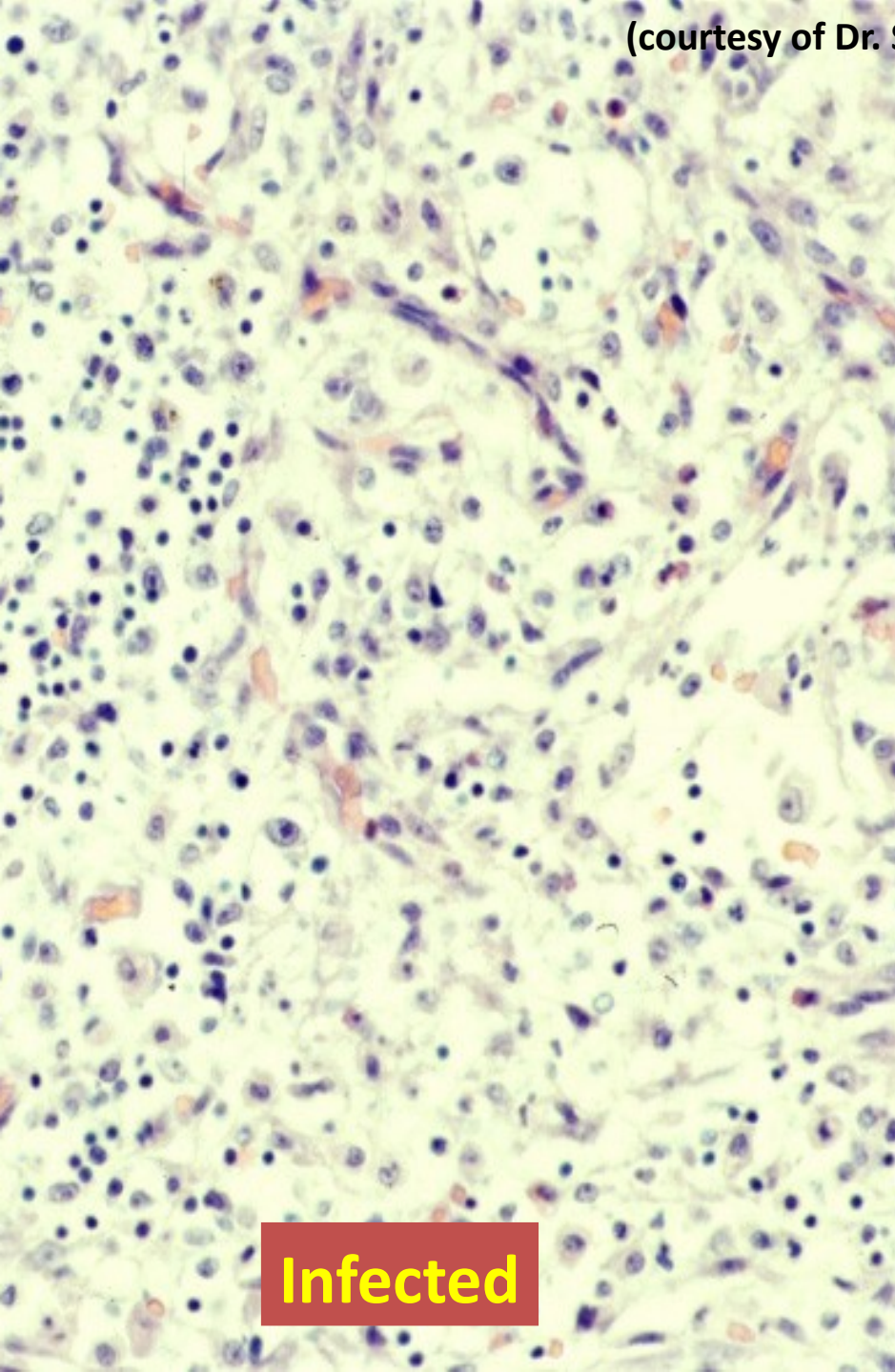


Fernandez *et al.*, 2008

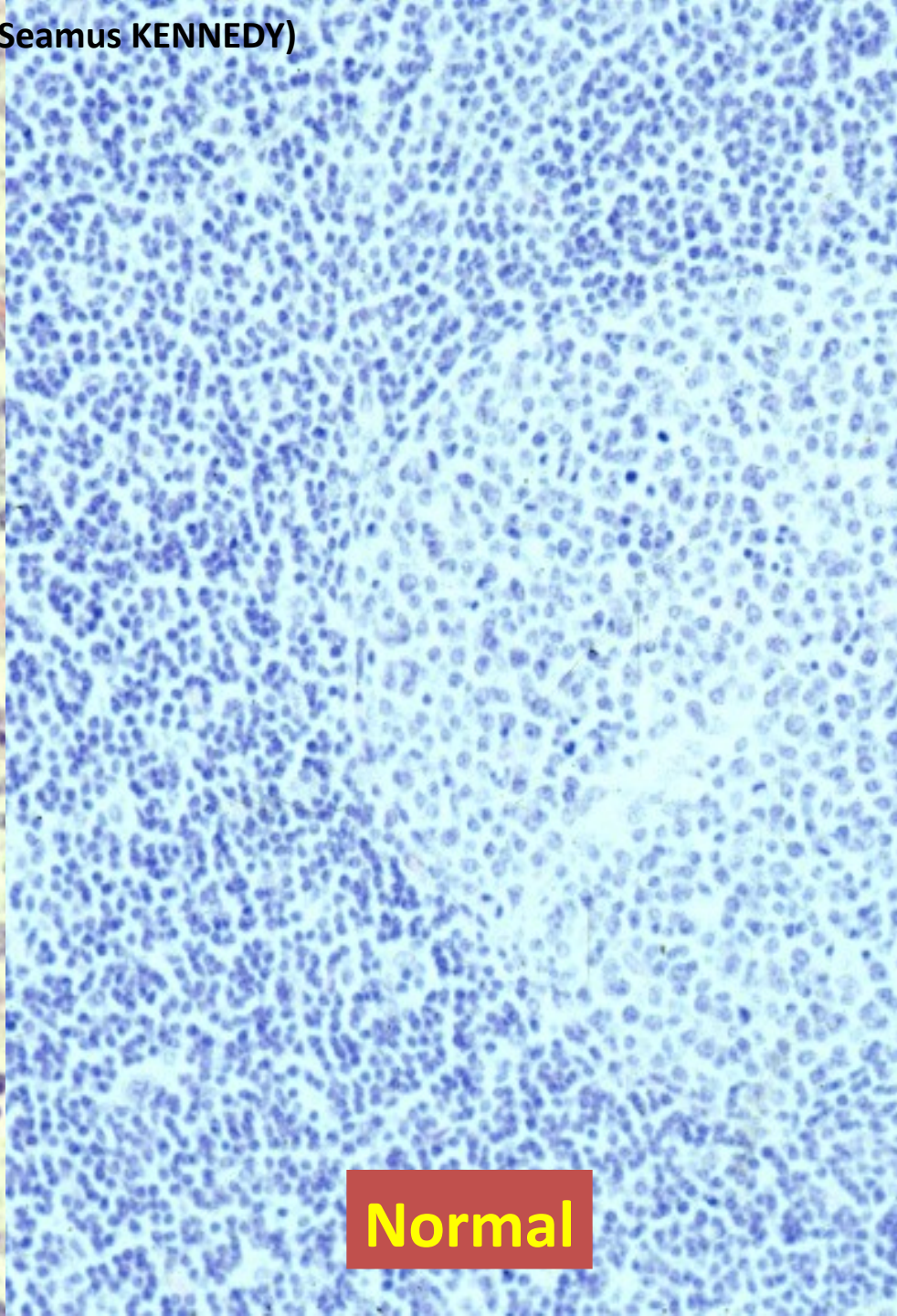
DMV Epidemics in the Western Mediterranean

2016, Mar Ionio (Puglia, Basilicata, Calabria e Sicilia), *Stenella coeruleoalba*; lesioni anatomico-istopatologiche «classiche/caratteristiche» con i «tipici» *patterns* di positività alle indagini immunoistochimiche; ceppo di DMV simile ad un isolato virale recentemente caratterizzato da Cetacei Odontoceti rinvenuti spiaggiati lungo le coste Atlantiche della Spagna e del Portogallo (Bento *et al.*, 2016; Casalone *et al.*, 2017, submitted for publication).

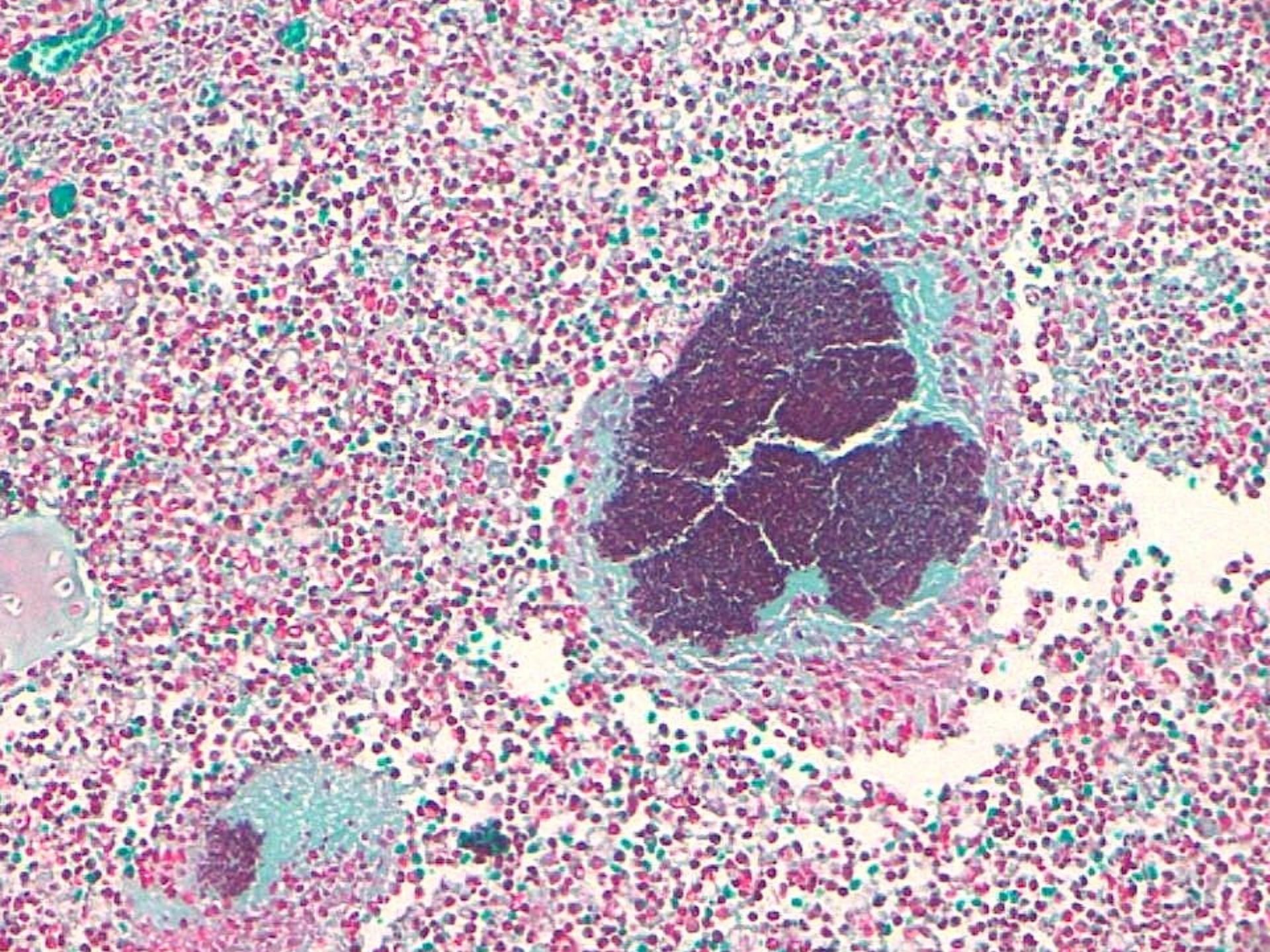
(courtesy of Dr. Seamus KENNEDY)

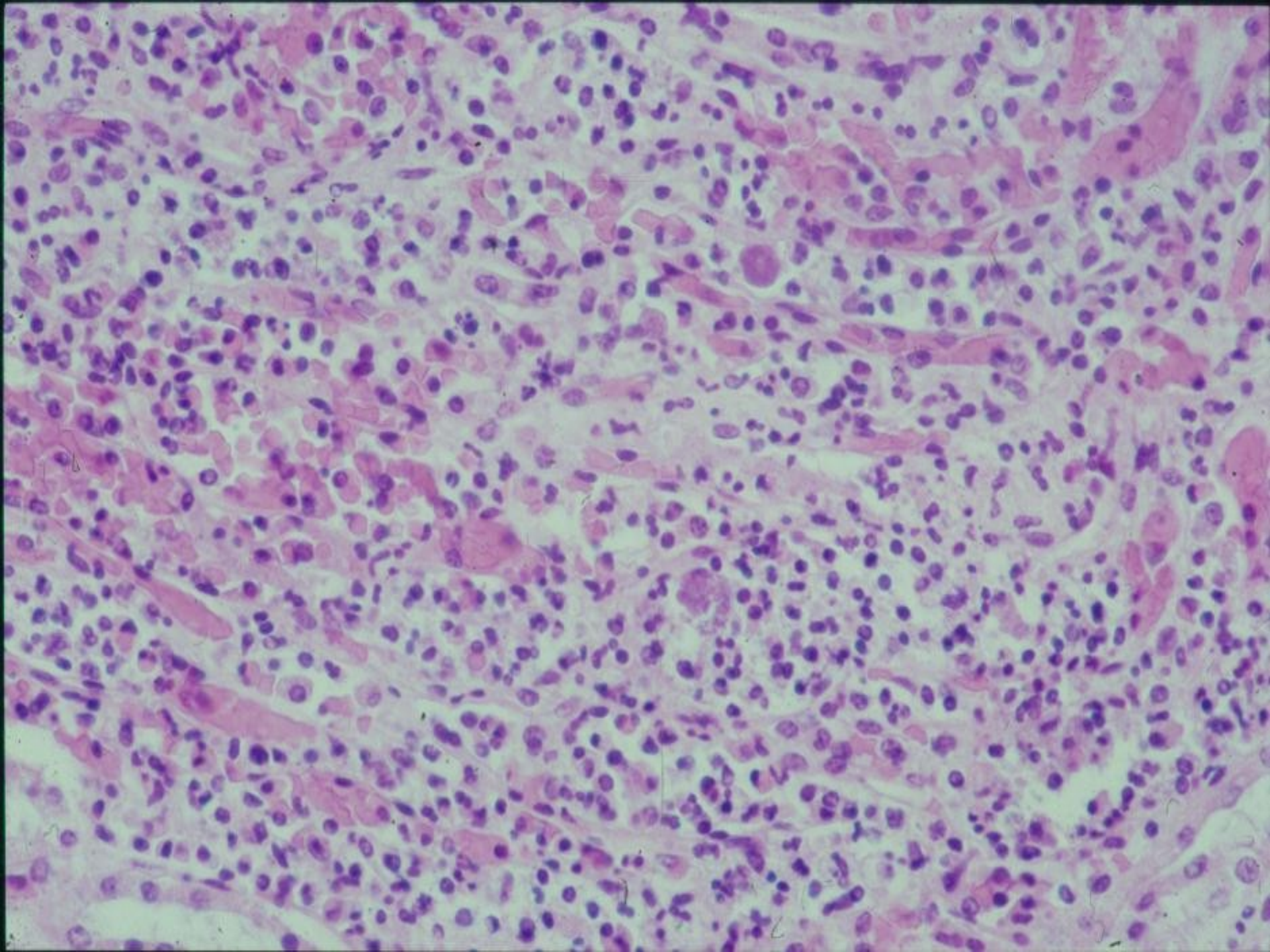


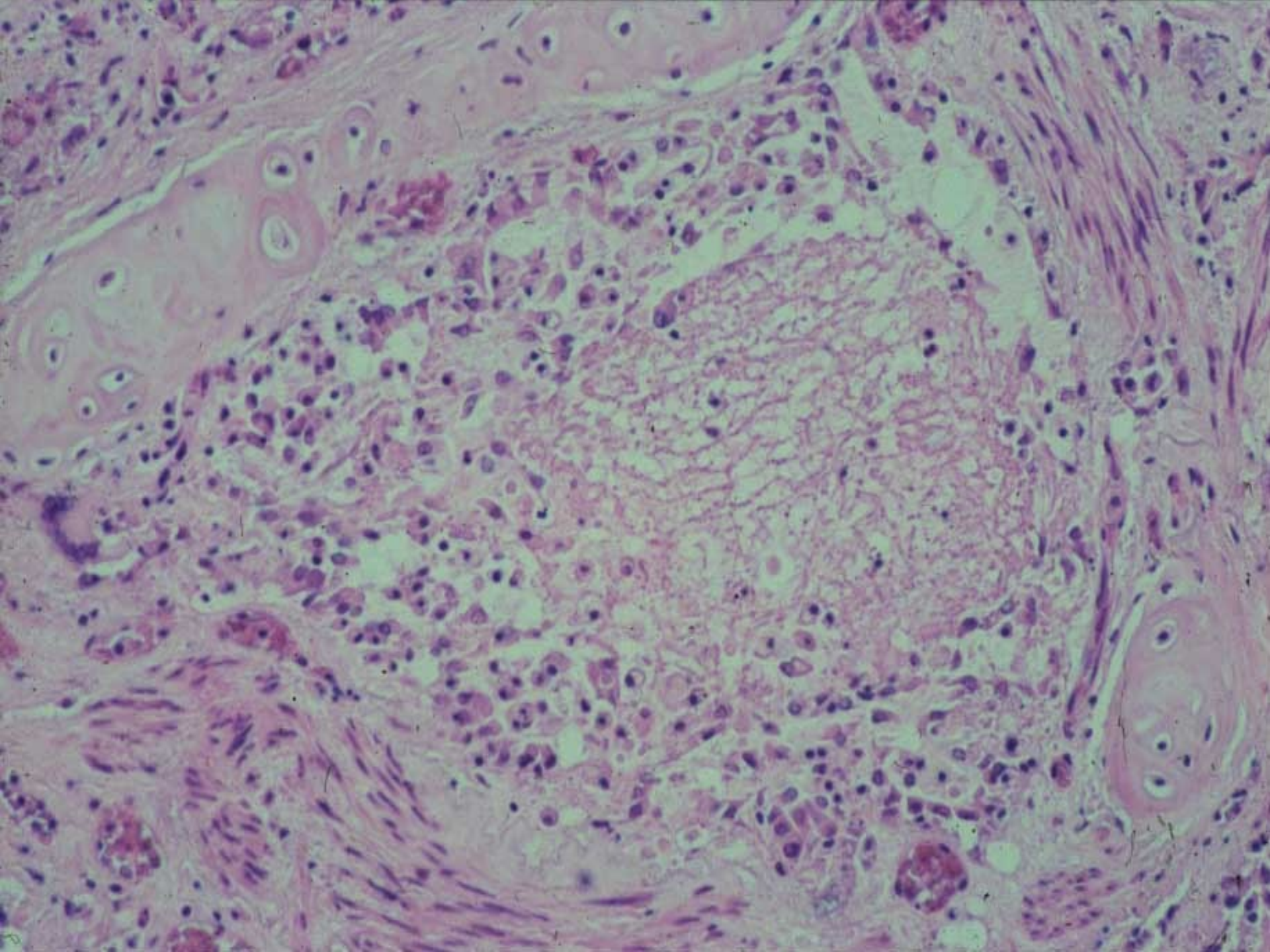
Infected

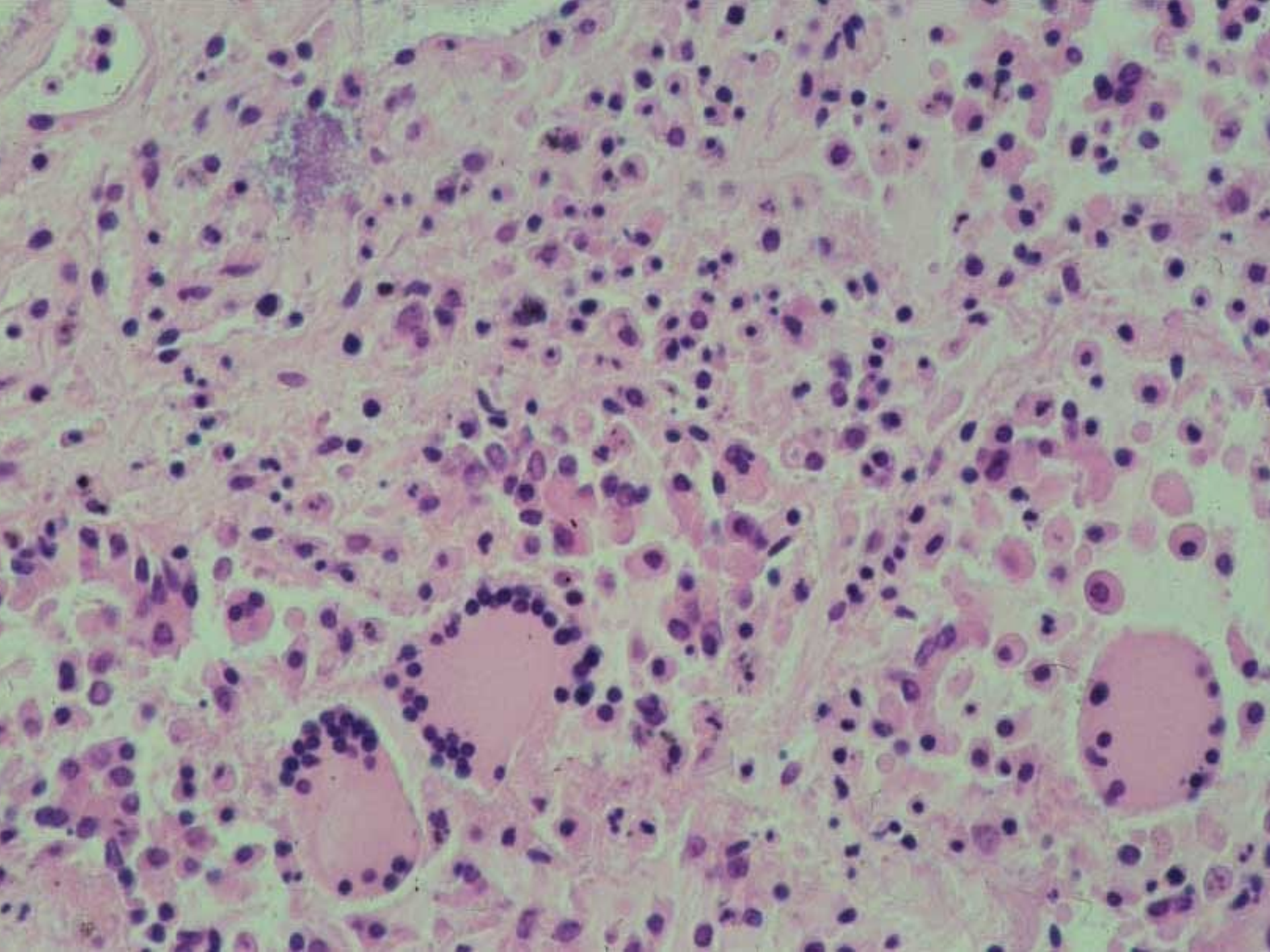


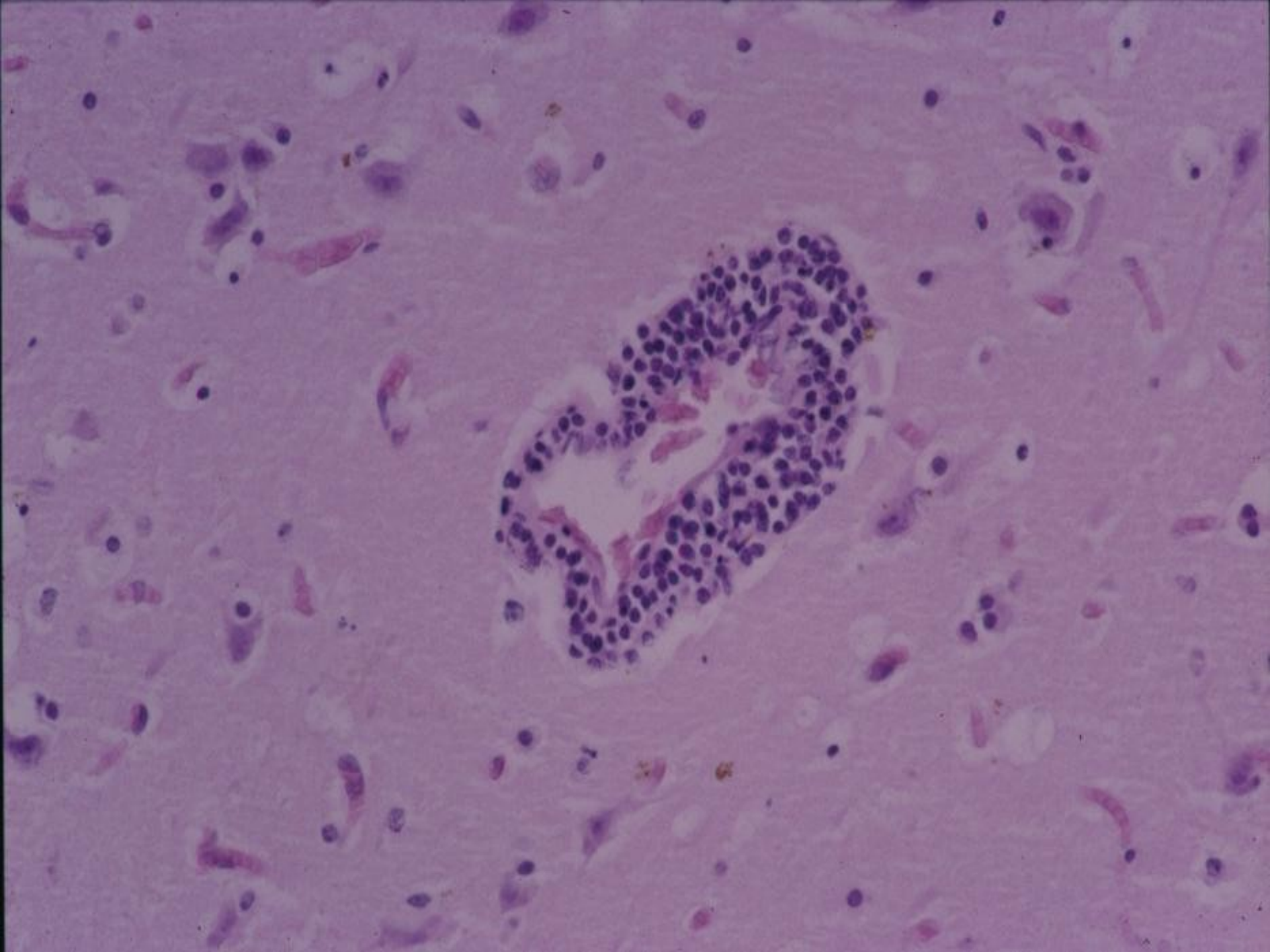
Normal

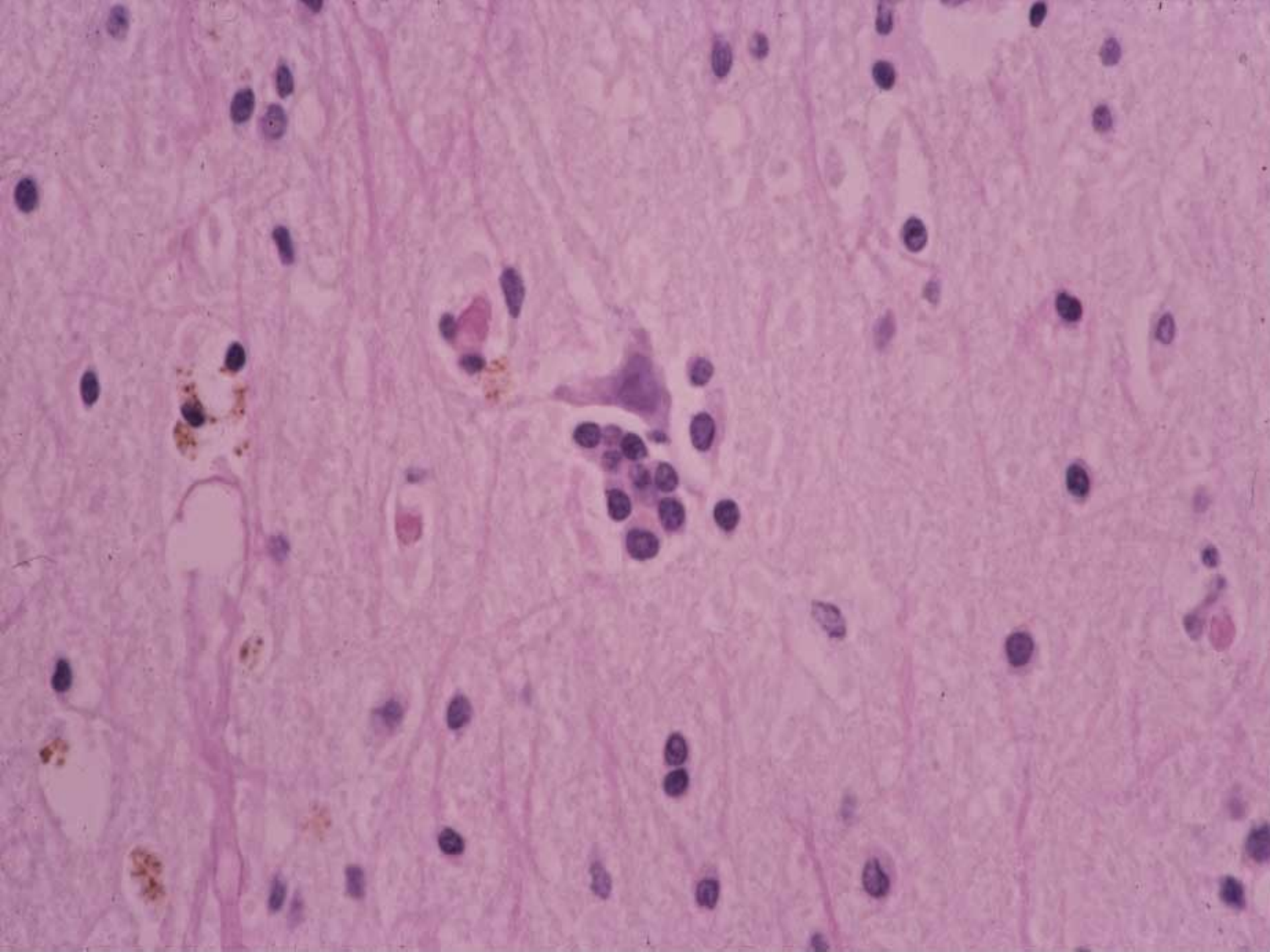


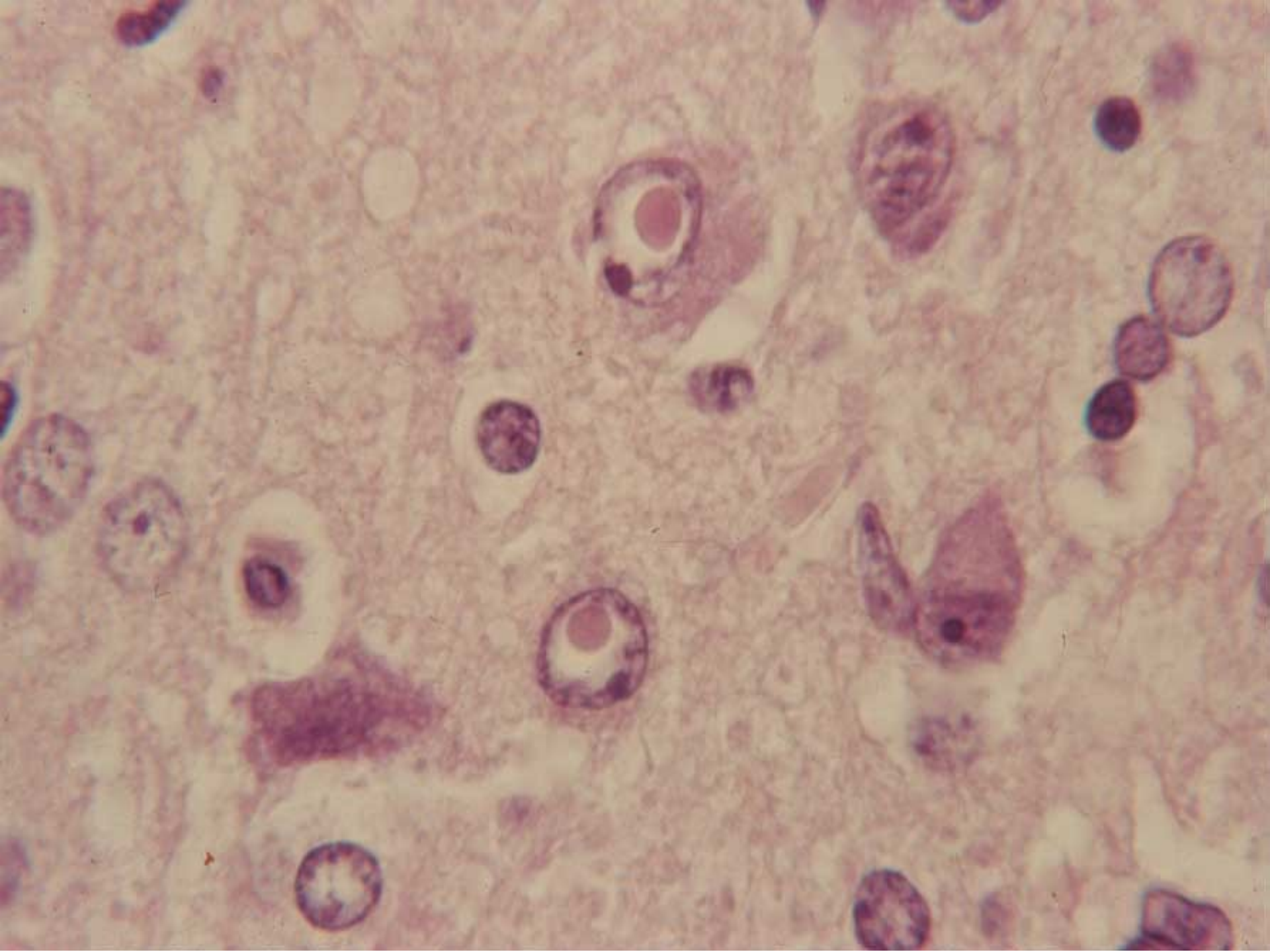


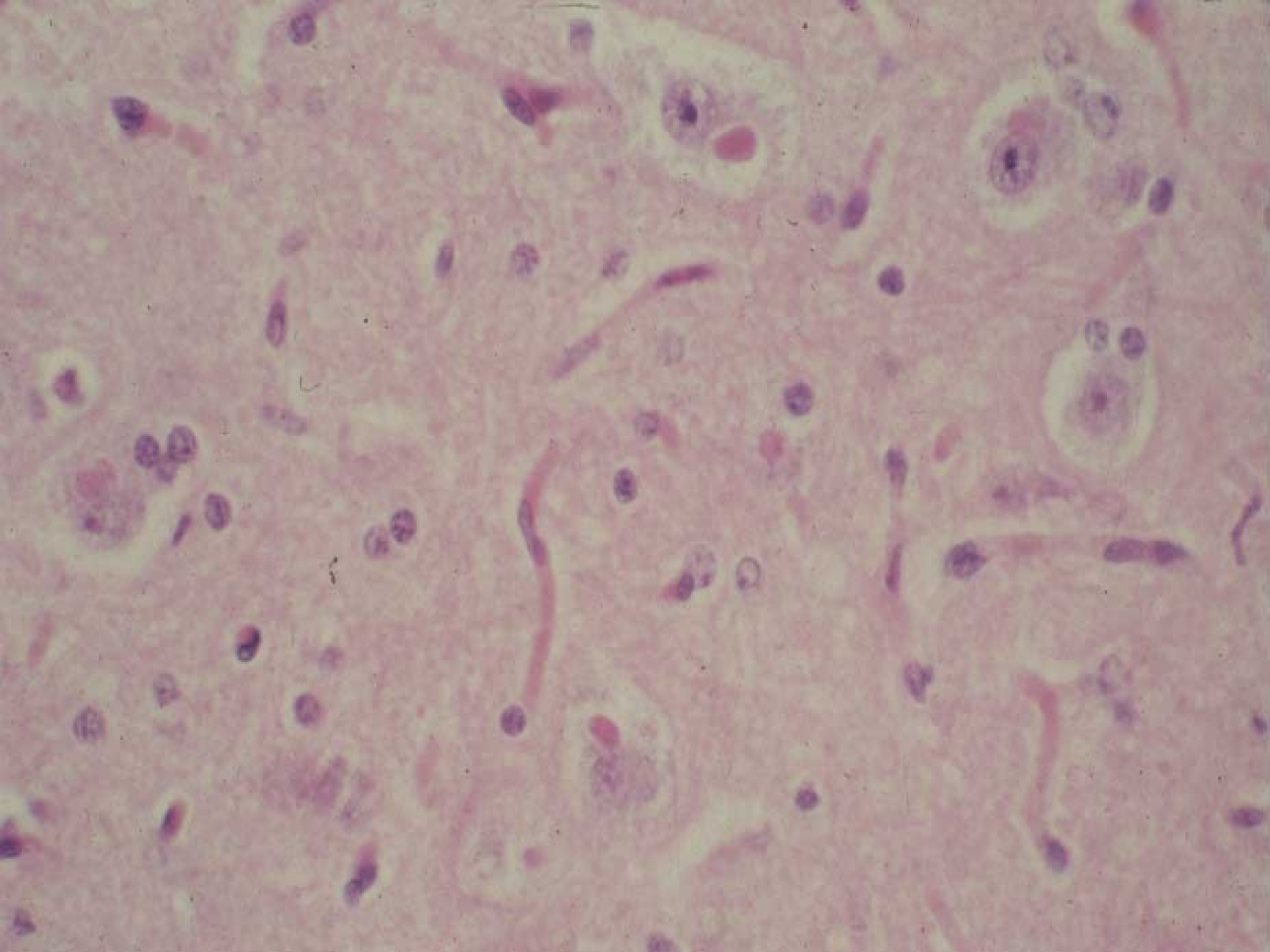


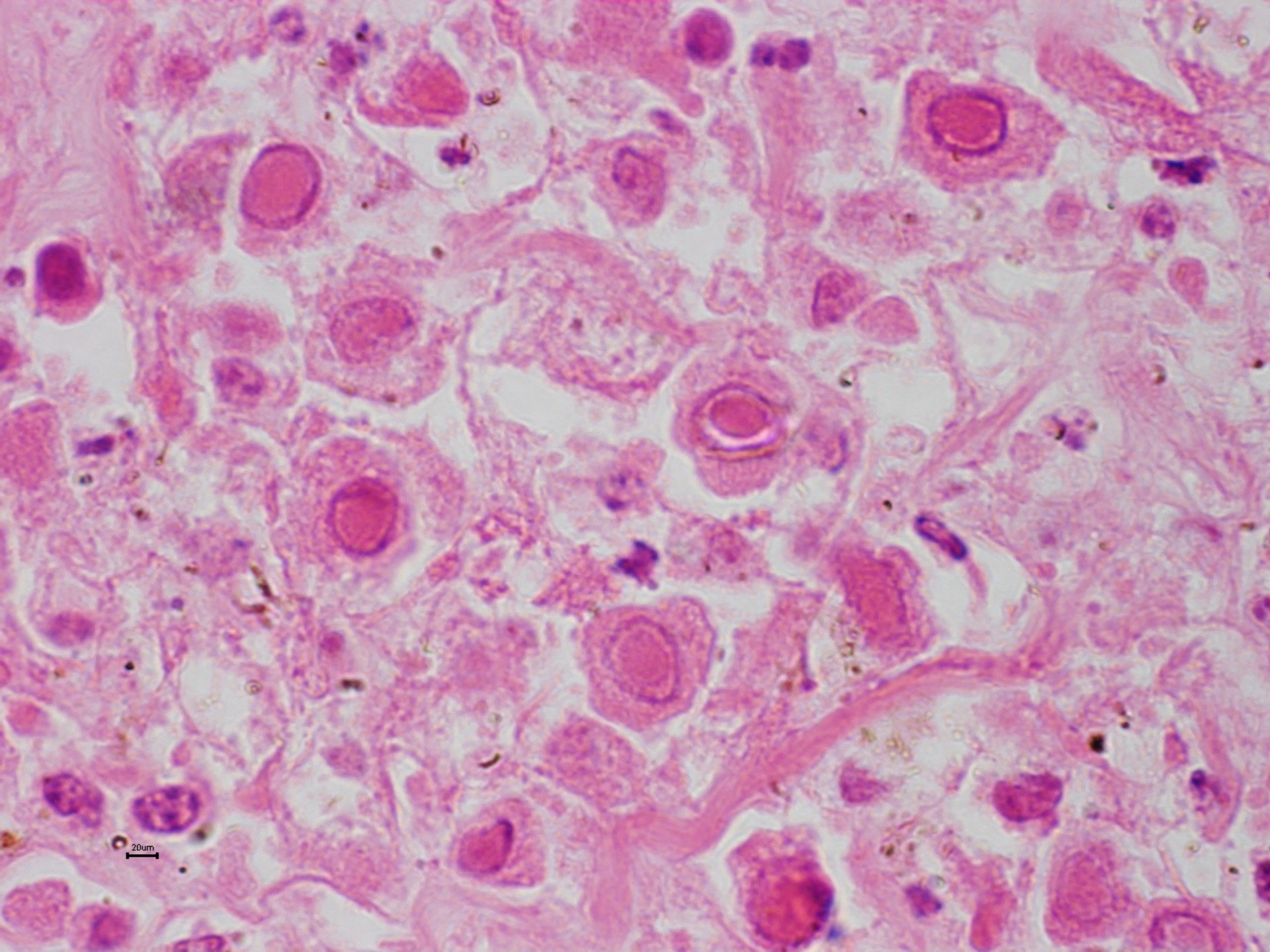


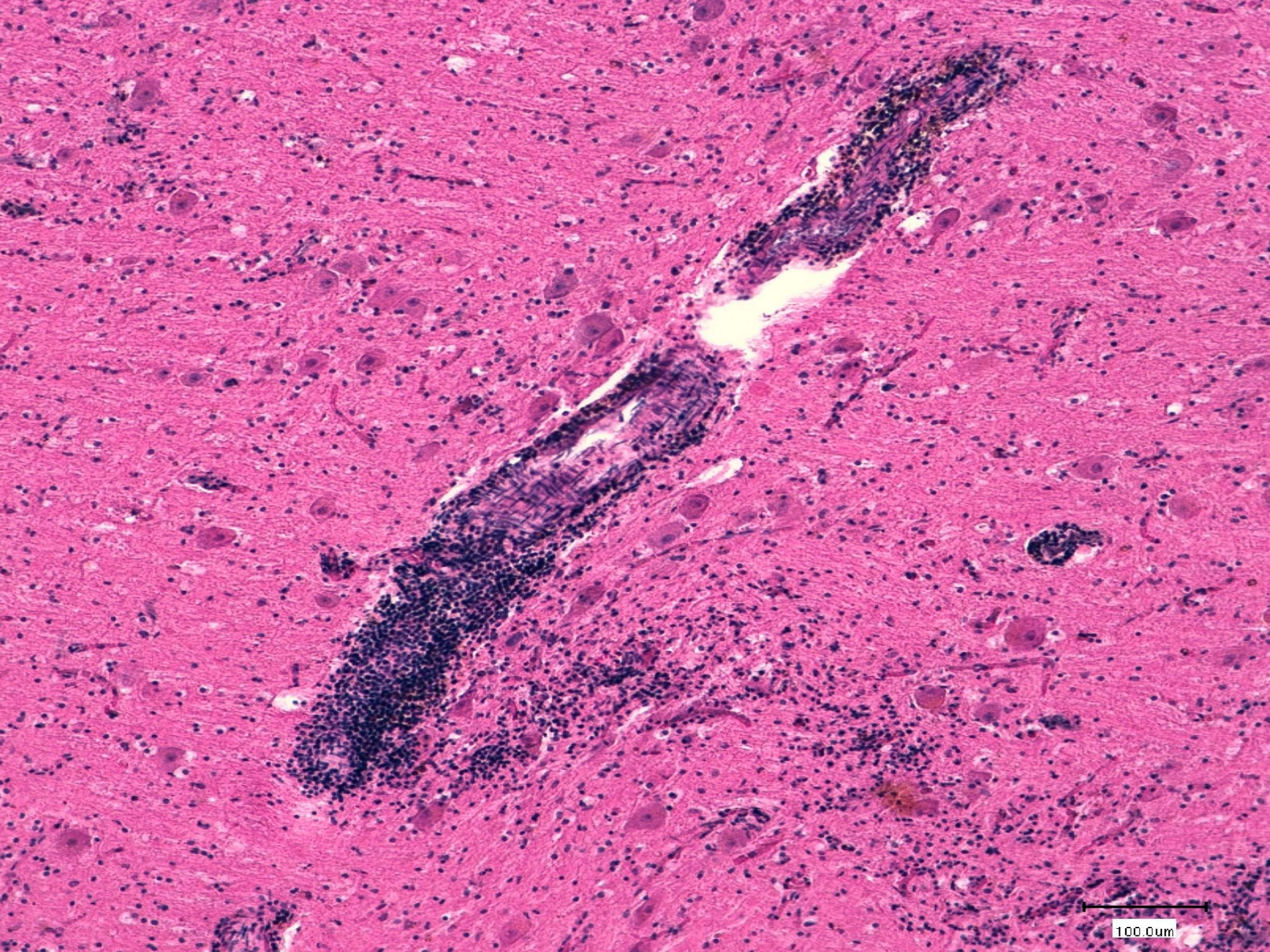


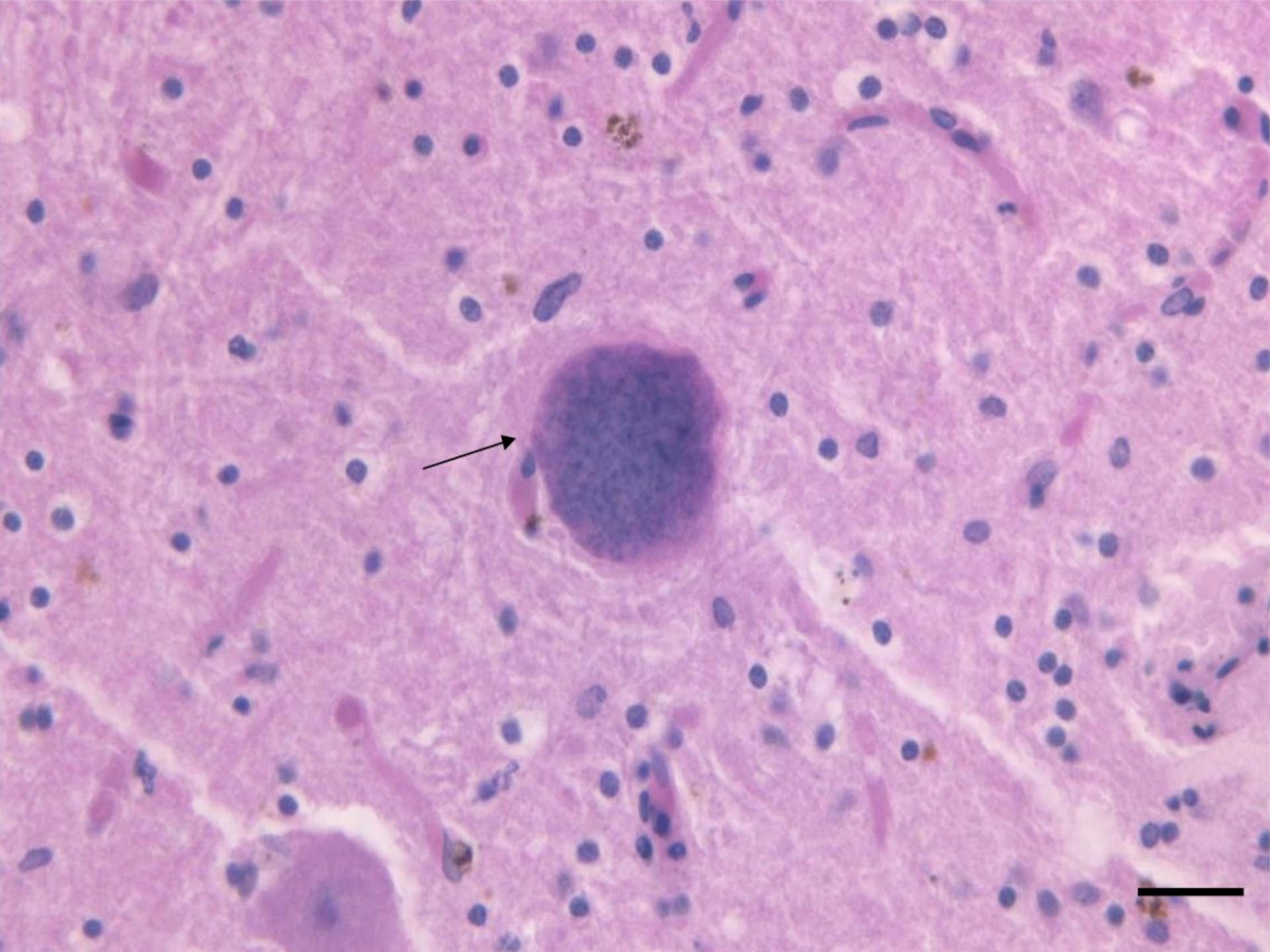














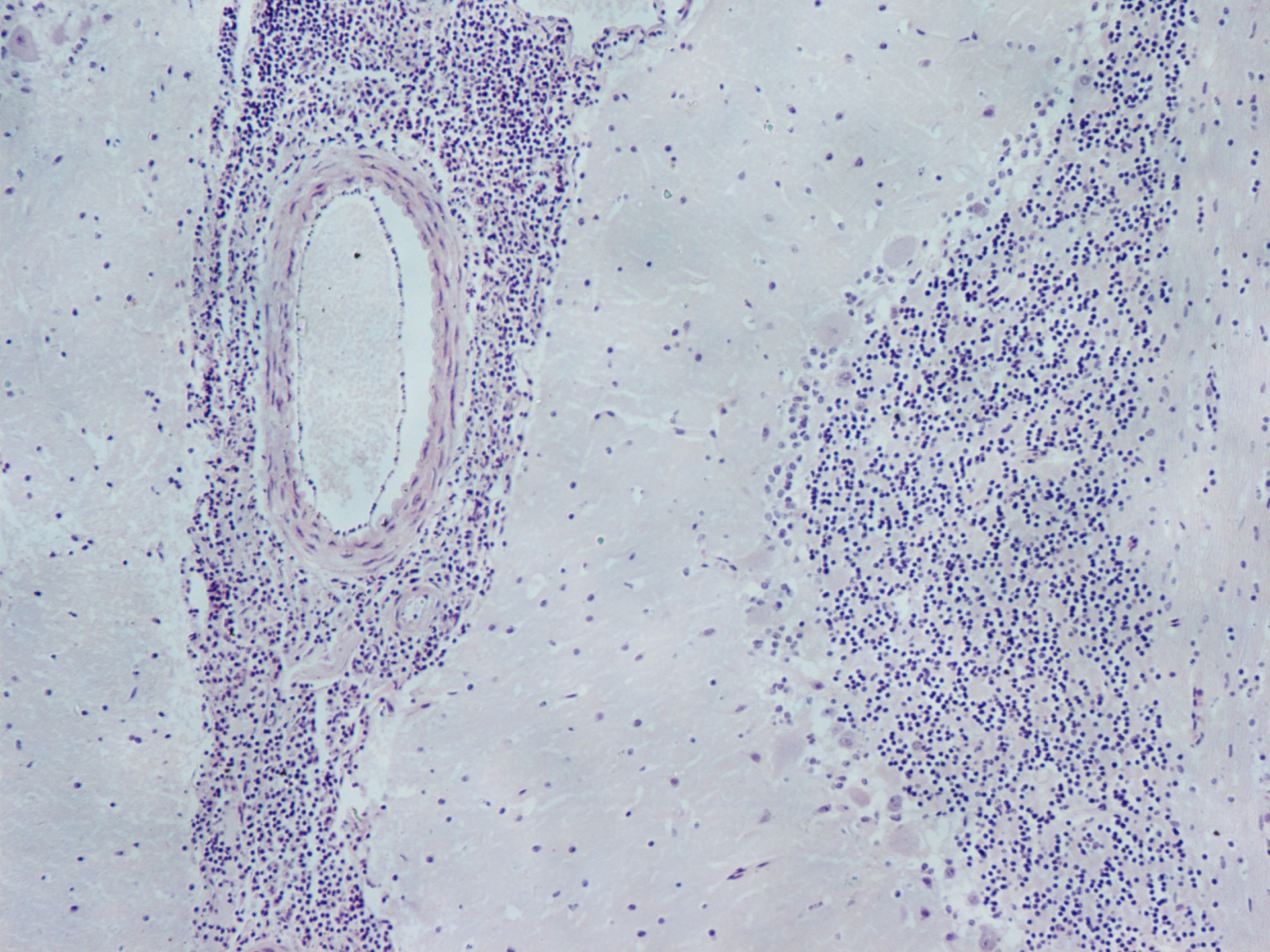
ITALY

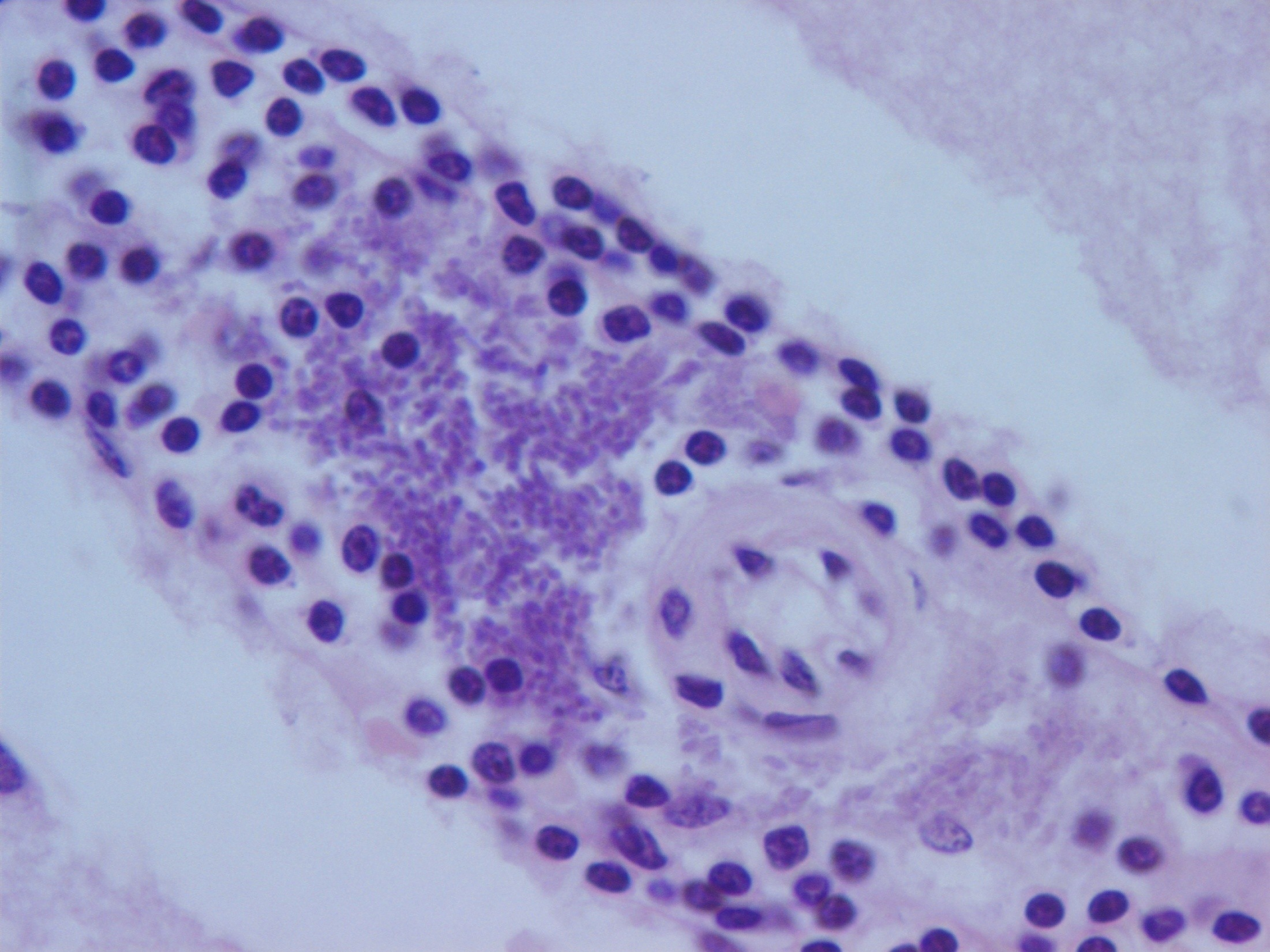
FRANCE

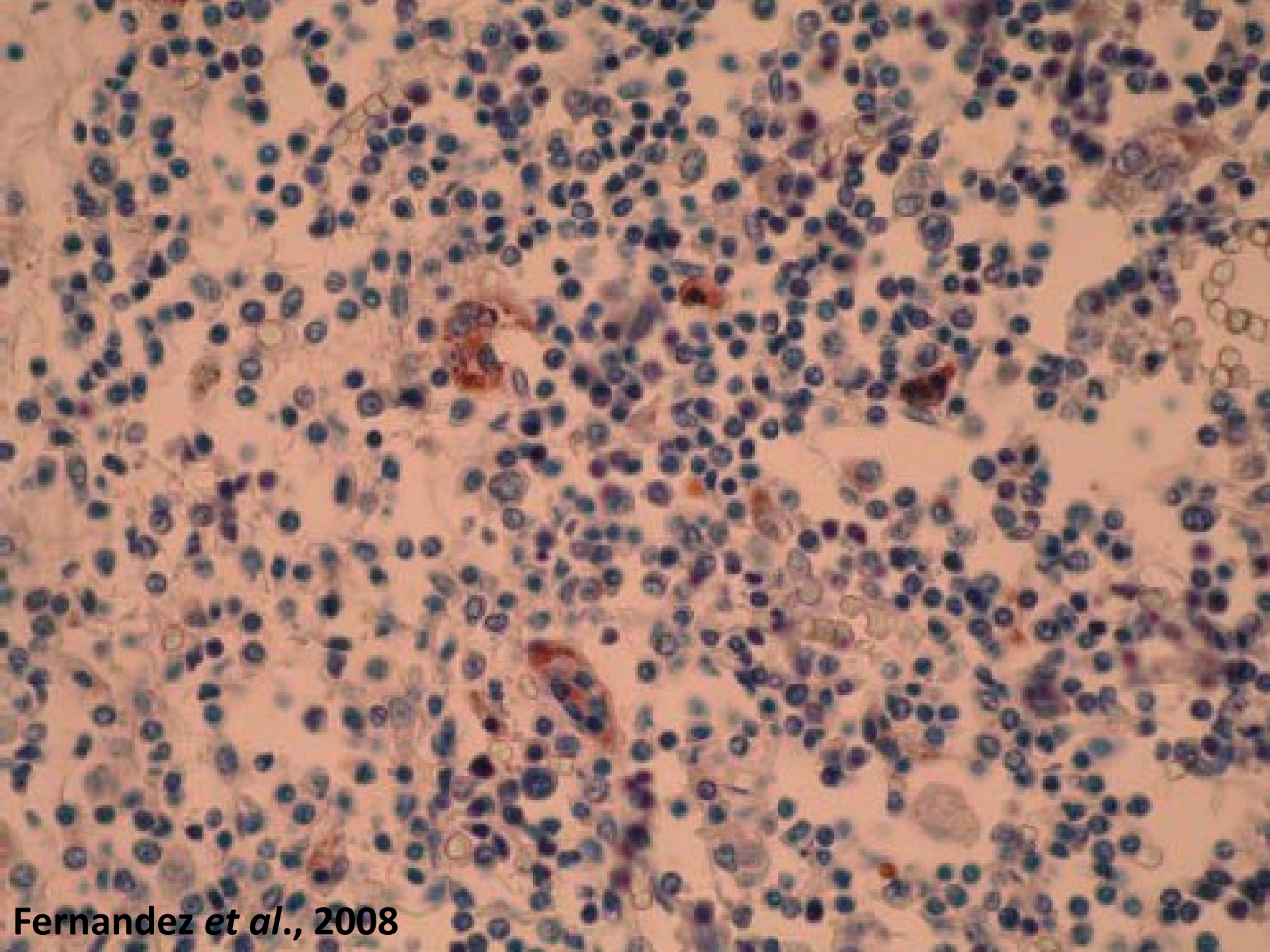
Imperia

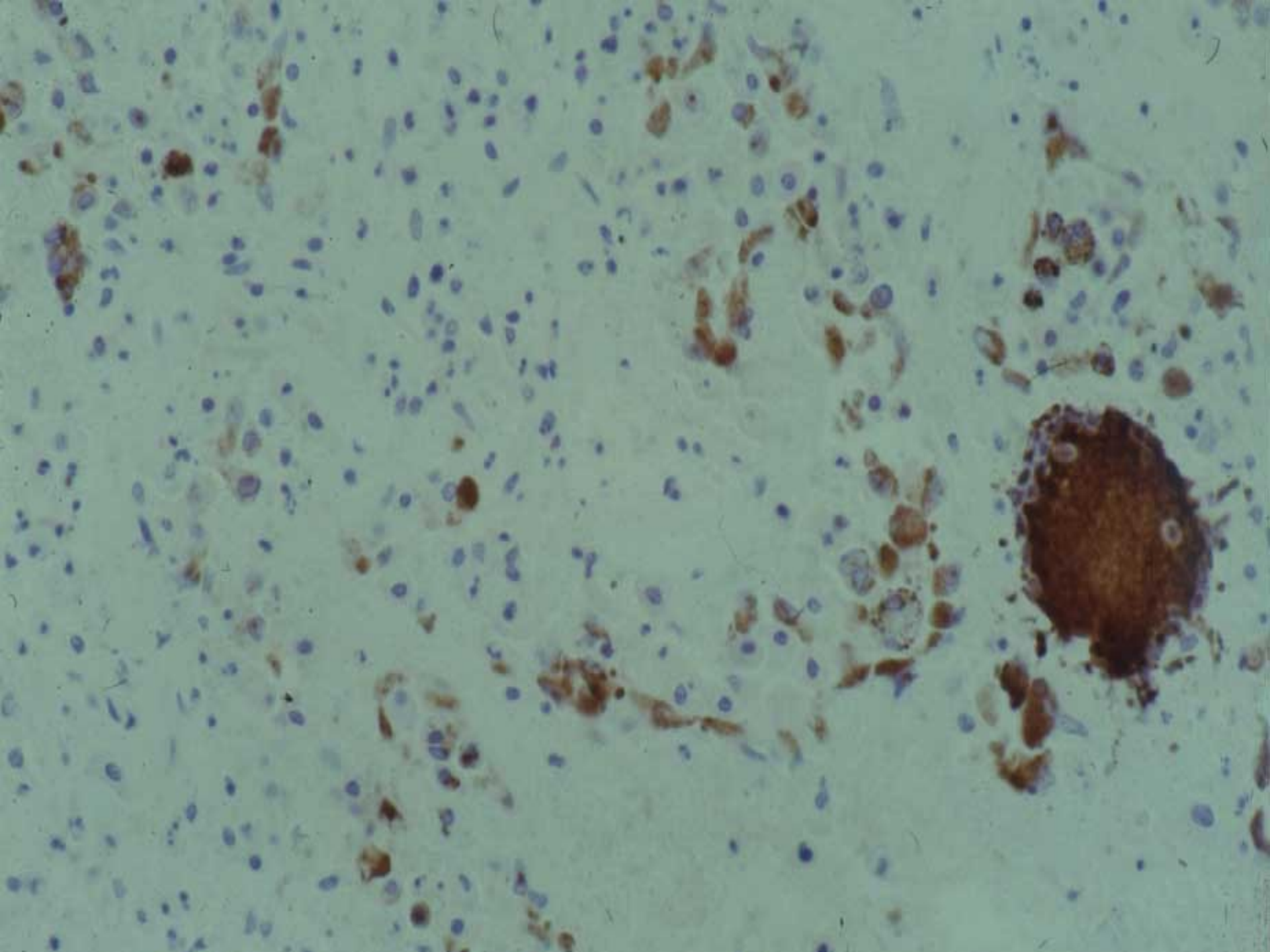
Ligurian Sea

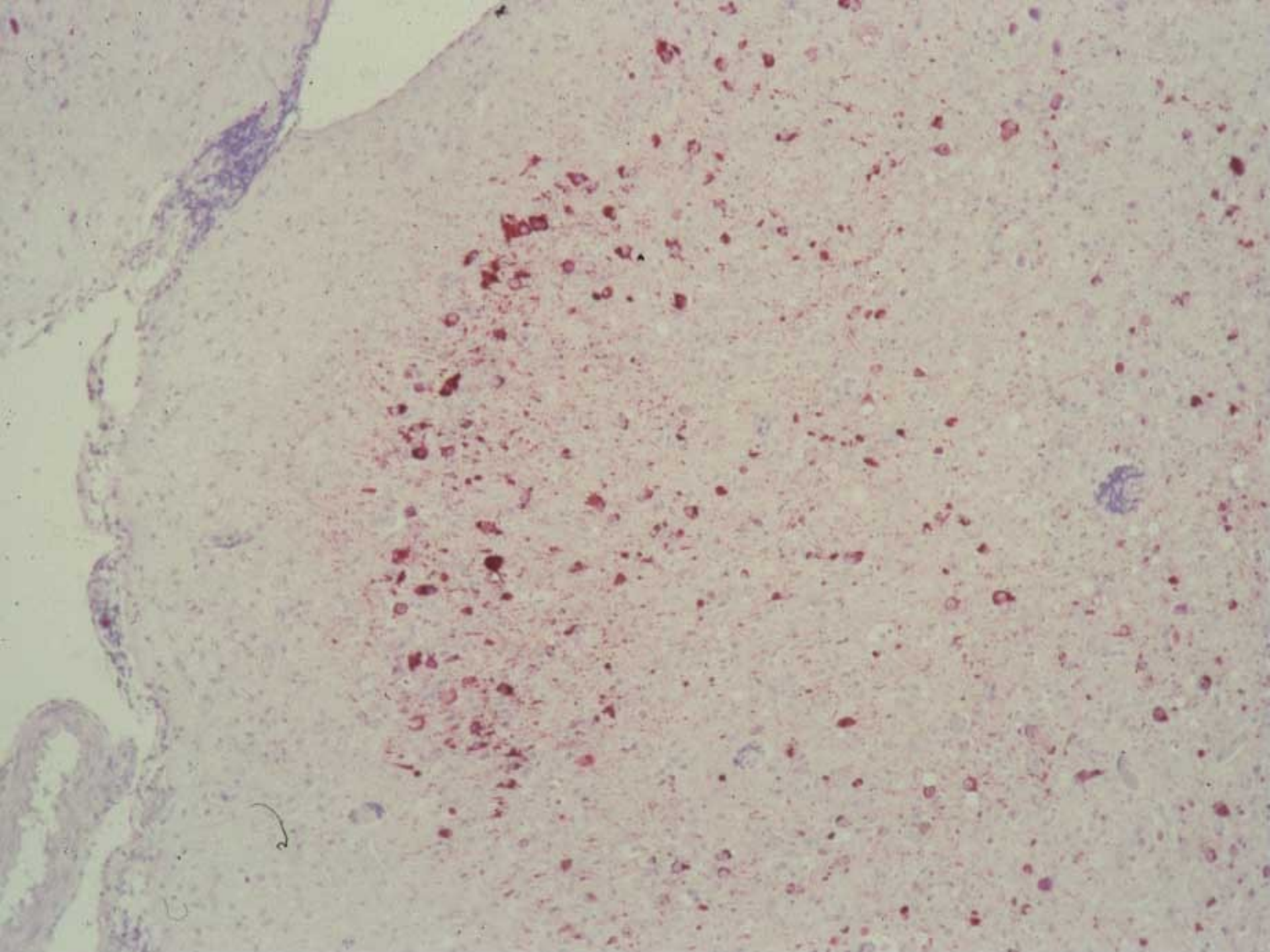


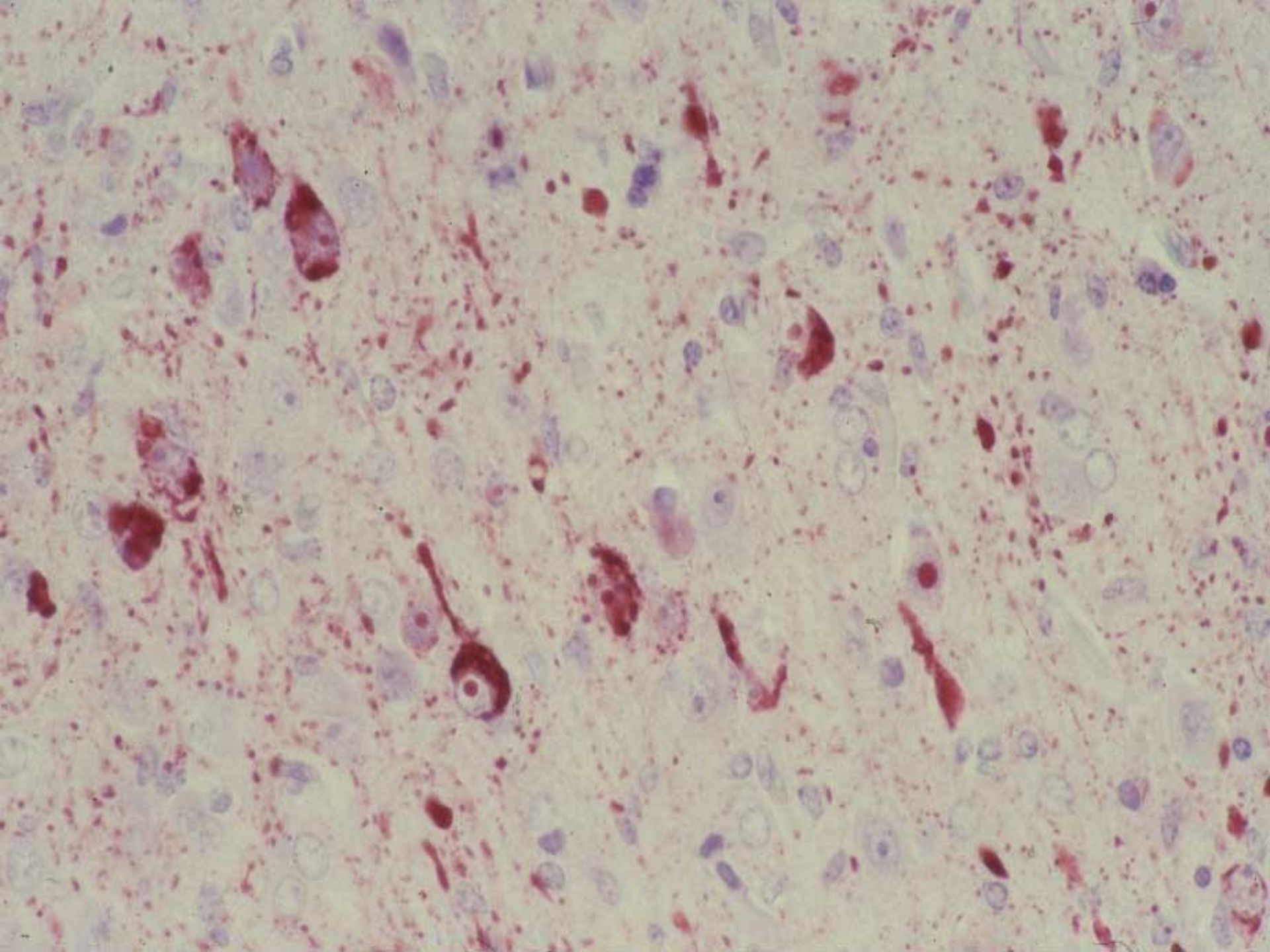


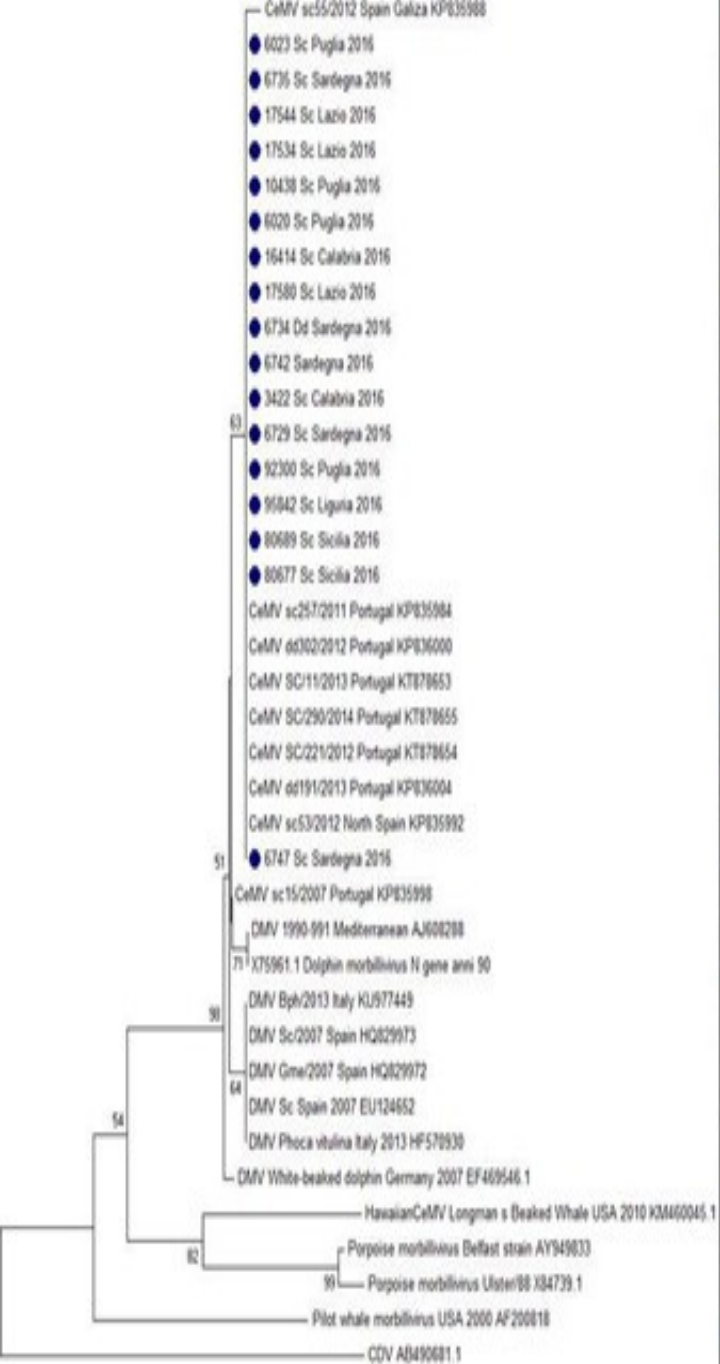




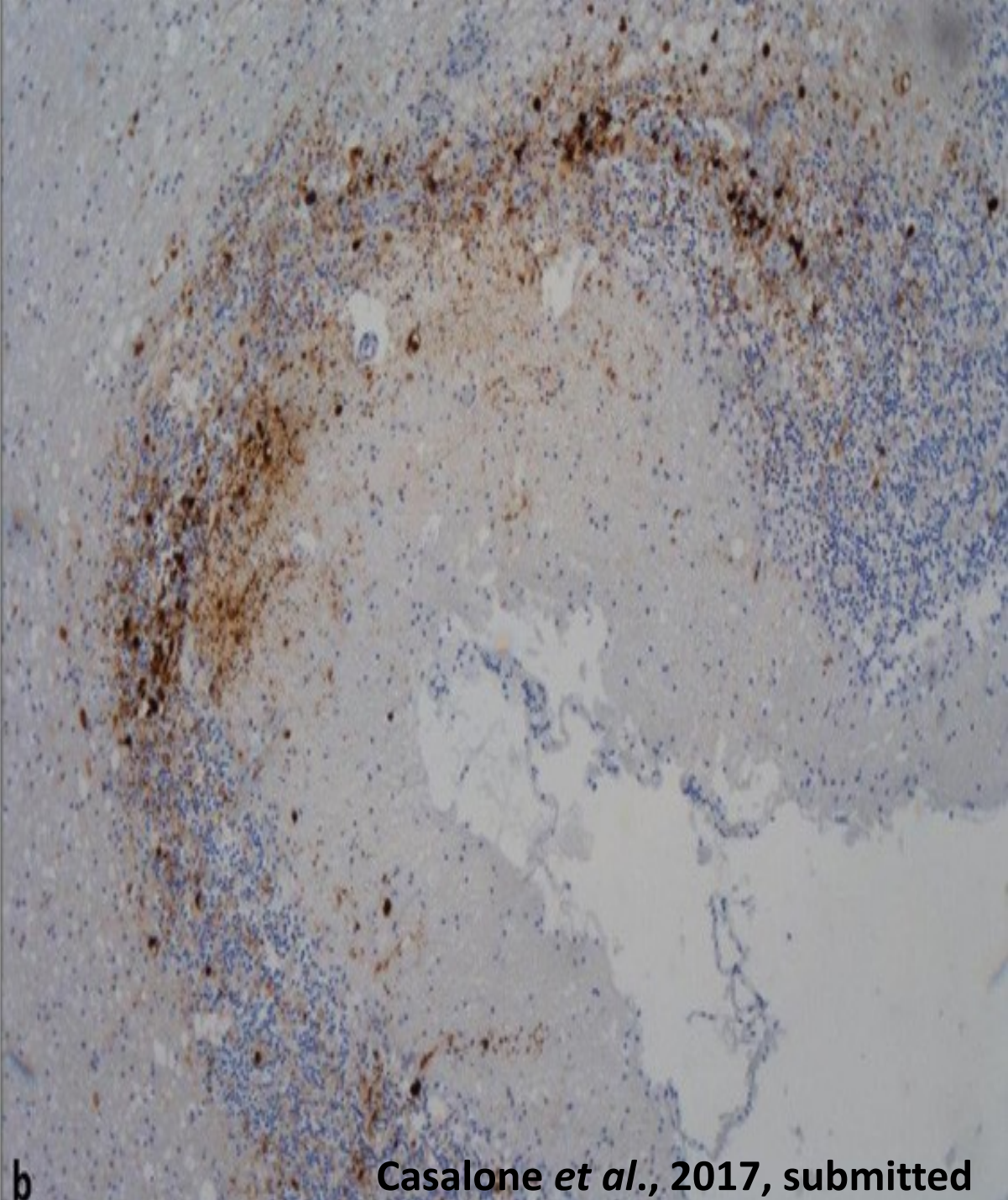






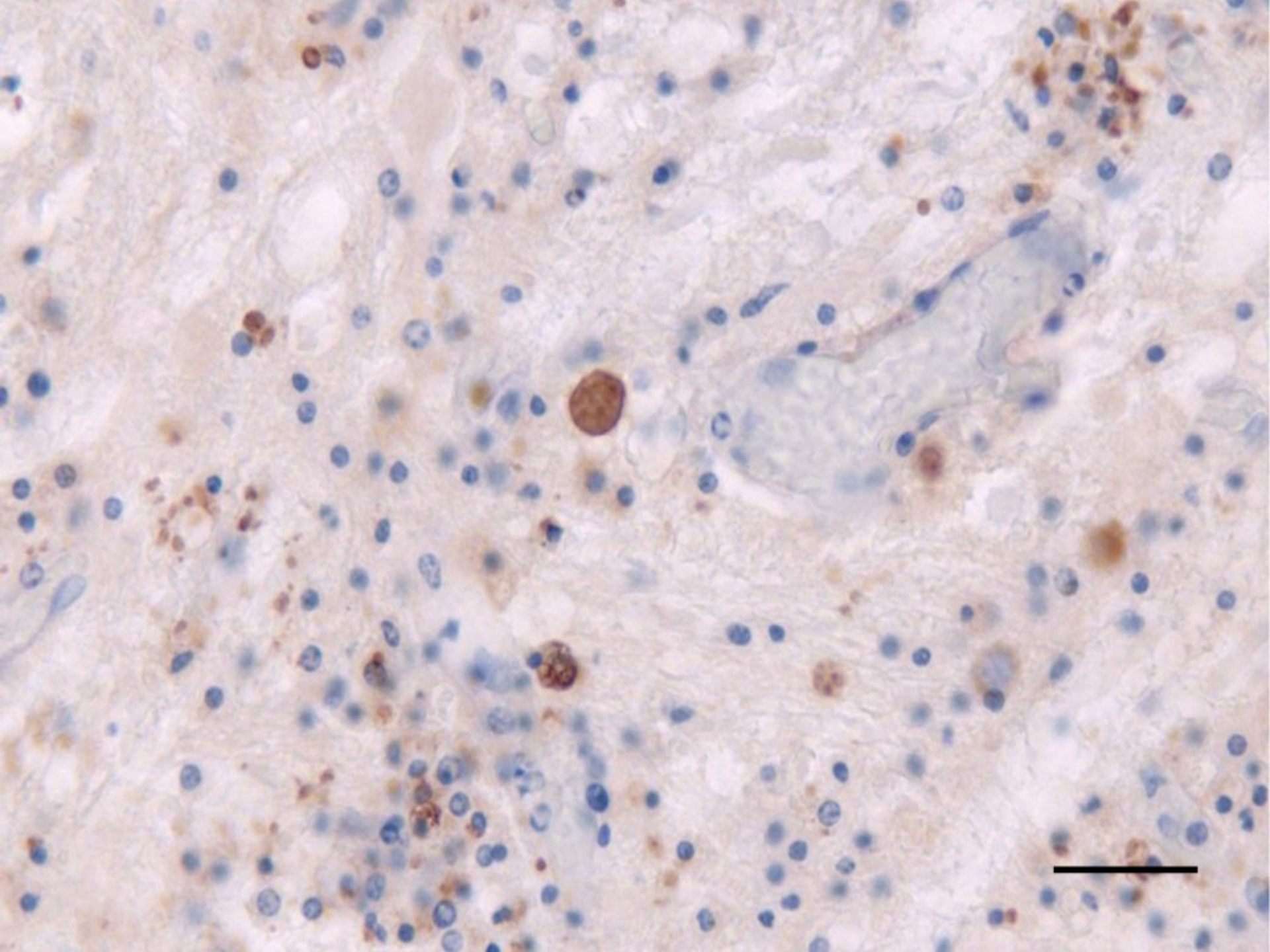


a 0.02



b

Casalone *et al.*, 2017, submitted



Monitoraggio sierologico

- *Screening* sierologico per *Morbillivirus*, *Brucella* spp., *Toxoplasma gondii* (Progetto di Ricerca *Sentinelle del Mare*, MATTM, 2013-'14)
- Indagini retrospettive su campioni di animali rinvenuti spiaggiati a partire dagli anni '90

Research in Veterinary Science 101 (2015) 89–92



Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/yrvsc



Short Communication

Retrospective seroepidemiological investigations against *Morbillivirus*, *Toxoplasma gondii* and *Brucella* spp. in cetaceans stranded along the Italian coastline (1998–2014)

Francesca Profeta^a, Cristina Esmeralda Di Francesco^a, Fulvio Marsilio^a, Walter Mignone^b, Fabio Di Nocera^c, Esterina De Carlo^c, Giuseppe Lucifora^c, Guido Pietroluongo^a, Marina Baffoni^a, Cristiano Cocomelli^d, Claudia Eleni^d, Giuliana Terracciano^d, Nicola Ferri^e, Gabriella Di Francesco^e, Cristina Casalone^b, Alessandra Pautasso^b, Sandro Mazzariol^f, Cinzia Centelleghes^f, Giovanni Di Guardo^{a,*}

^a University of Teramo, Faculty of Veterinary Medicine, Teramo, Italy

^b Istituto Zooprofilattico Sperimentale (IZS) del Piemonte, Liguria e Valle d'Aosta, Italy

^c IZS del Mezzogiorno, Italy

^d IZS delle Regioni Lazio e Toscana "M. Aleandri", Italy

^e IZS dell'Abruzzo e del Molise "G. Caporale", Italy

^f Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro (PD), Italy



established in 1993

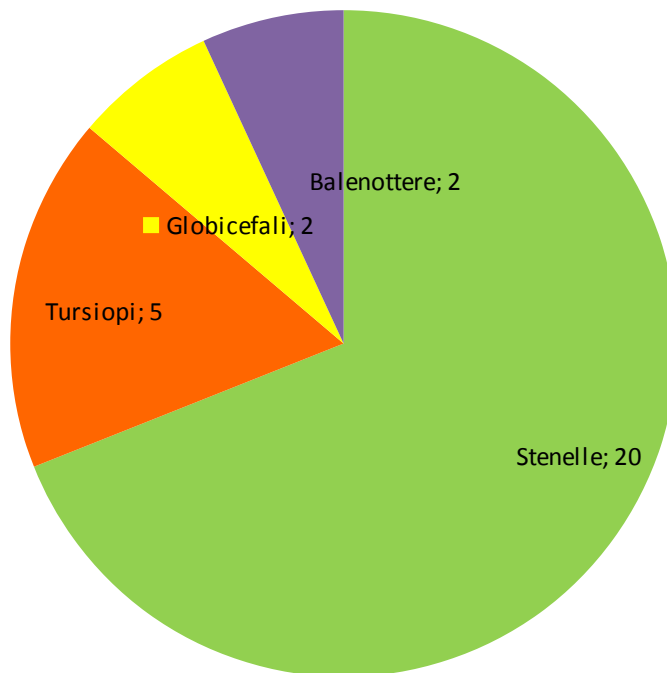
Marine Mammals' Serum Bank



MINISTERO DELL'AMBIENTE
E DELLA TUTELA DEL TERRITORIO E DEL MARE

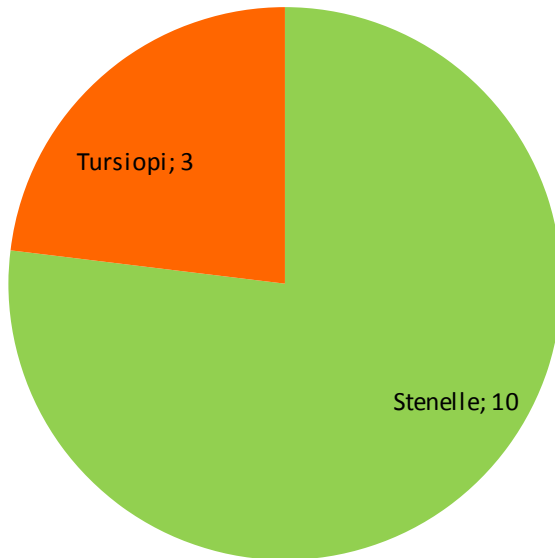
RISULTATI - *Morbillivirus*

- ✓ 29 campioni positivi (37,17%), con titoli anticorpali variabili da 1:10 a 1:64



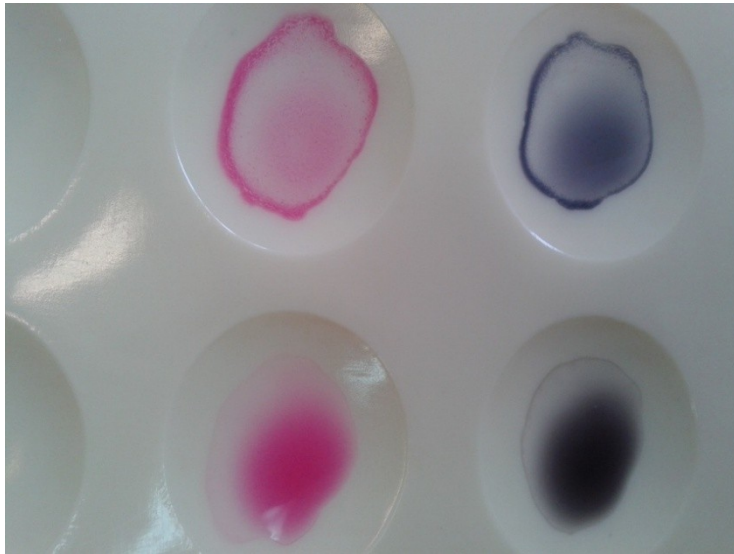
RISULTATI - *T. gondii*

- ✓ 13 campioni positivi (16,66%), con titoli anticorpali compresi tra 1:80 e $\geq 1:2560$.



RISULTATI - *Brucella* spp.

Brucella spp.: 1 campione positivo ottenuto da una stenella striata spiaggiatasi a Finale Ligure ad inizio 2015



← Campione positivo

← Campione negativo

Cetacean Morbillivirus (CeMV): Host range

1. Dolphin Morbillivirus (DMV)



Tursiops truncatus



Stenella coeruleoalba



Delphinus delphis ponticus
???

2. Porpoise Morbillivirus (PMV)



Phocoena phocoena

3. Pilot Whale Morbillivirus (PWMV)



Globicephala melas

4. Beaked Whale Morbillivirus (BWMV)

5. Guiana Dolphin Morbillivirus

6. Indo-Pacific Bottlenose Dolphin Morbillivirus

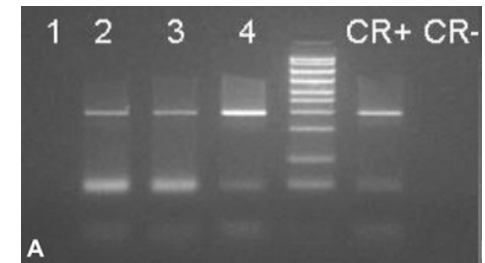
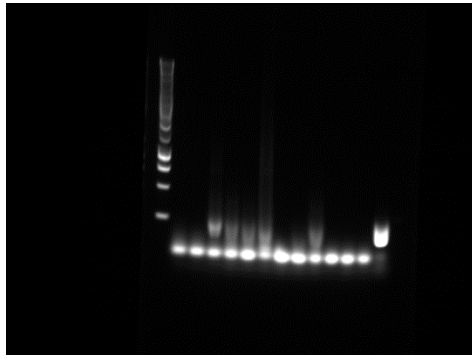


CeMV clade?

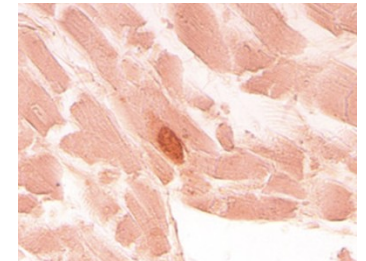
DMV in *Balaenoptera physalus*



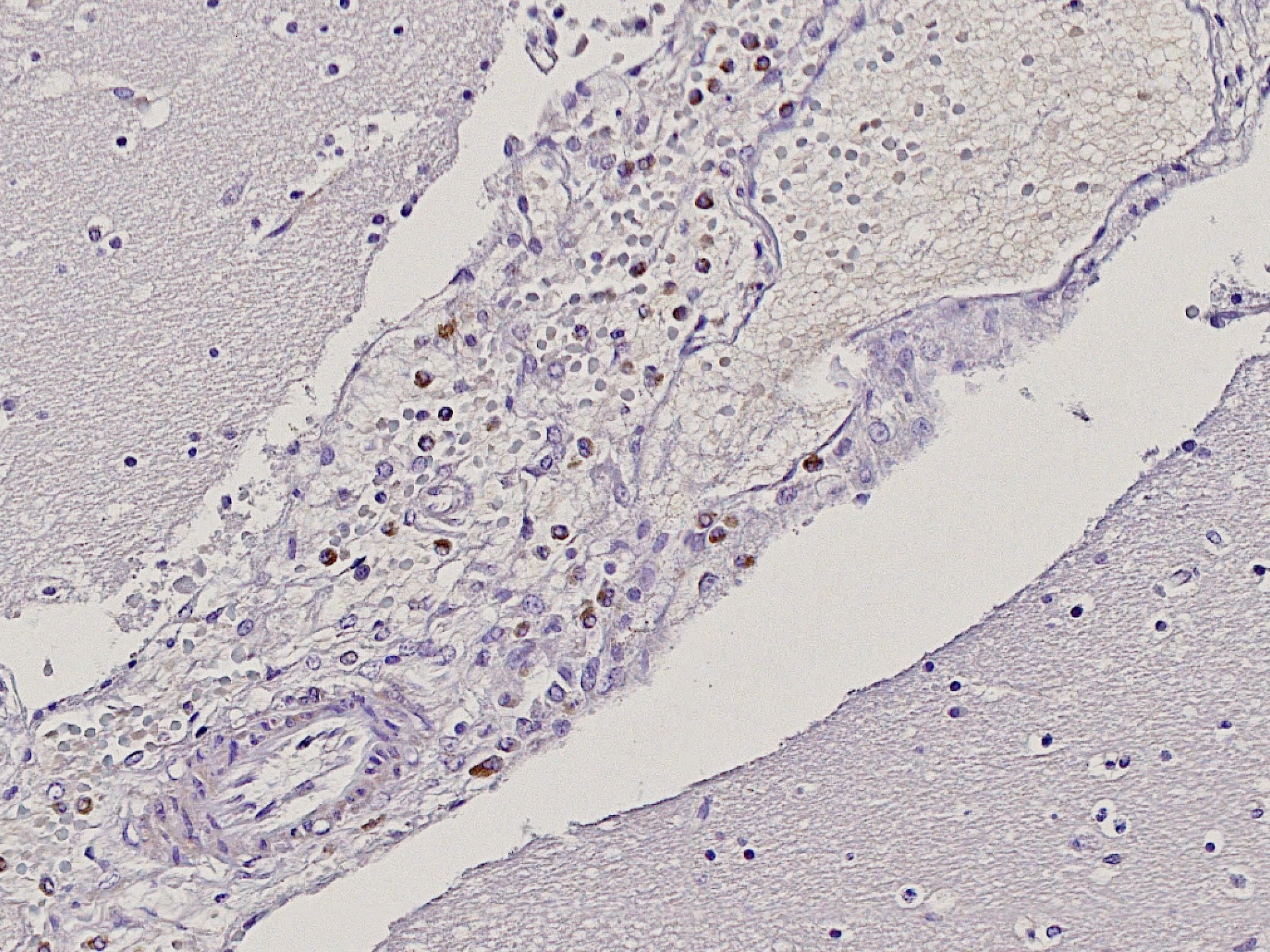
- 4 casi (Toscana, Sardegna, Liguria e Isola d'Elba)
- 2011-2013
- Trasmissione verticale in un individuo

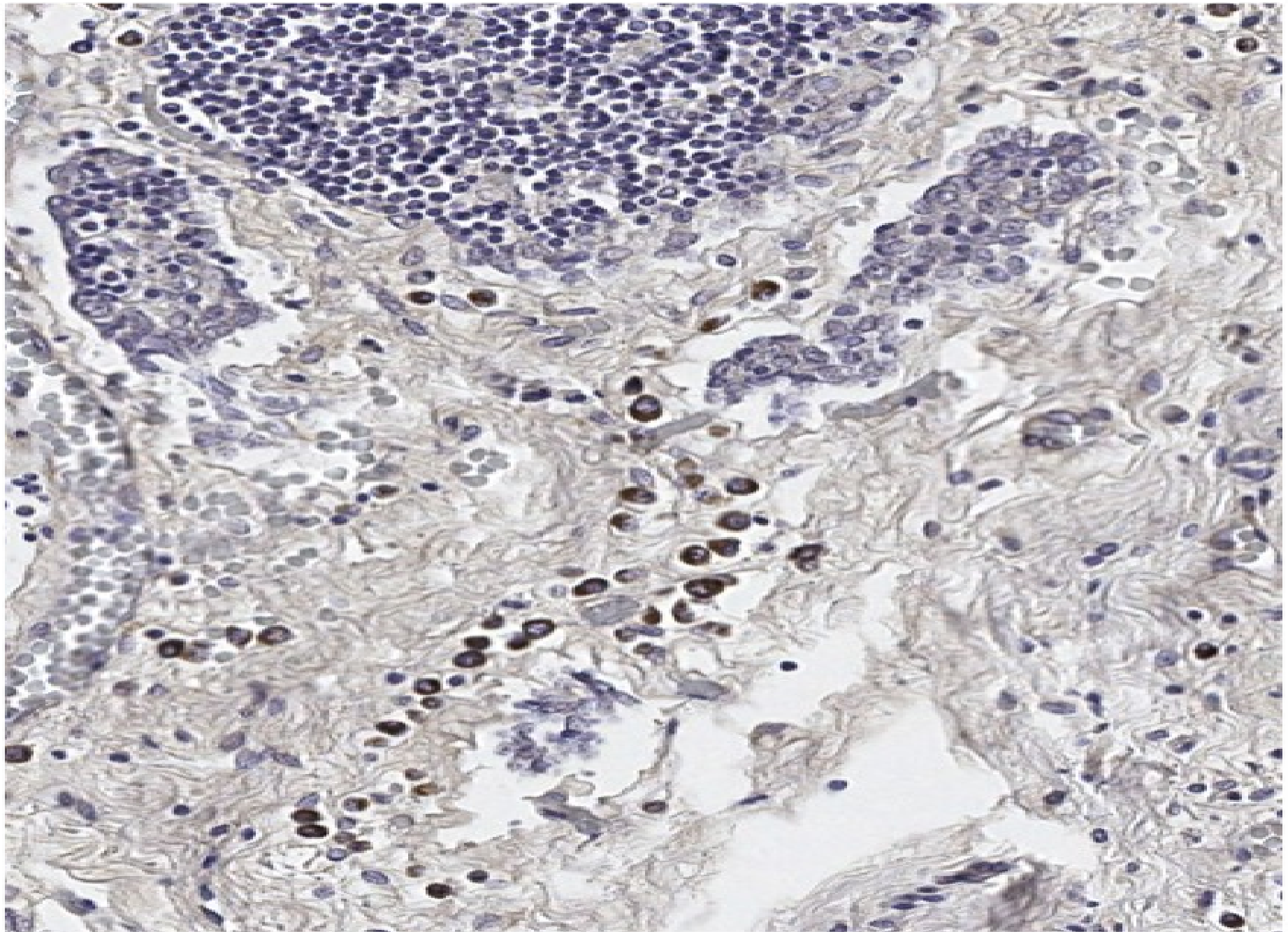


Mazzariol e Di Guardo, 2014

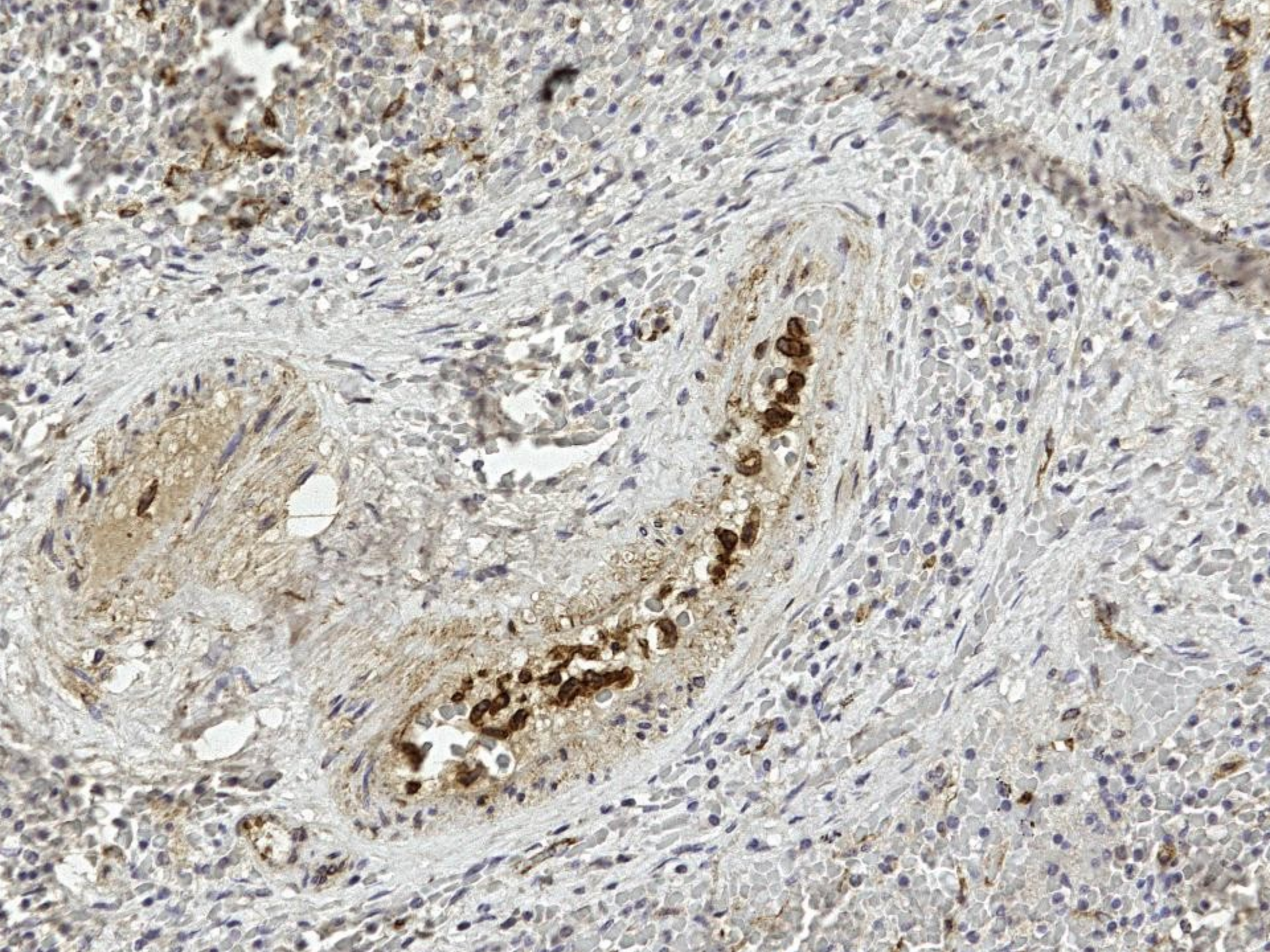


Mazzariol *et al.*, 2012



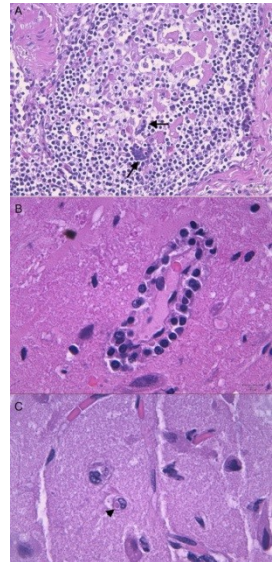
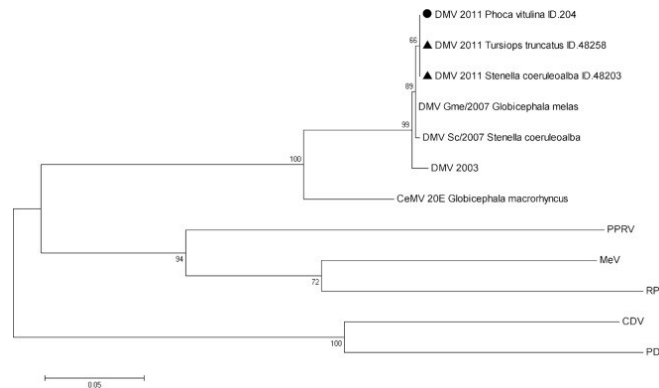






DMV in *Phoca vitulina*

- Esemplare in cattività
- Trasmissione tra cetacei e pinnipedi
- Pregressa epidemia (?) da MSMV in *Monachus monachus* (Mauritania, 1997)

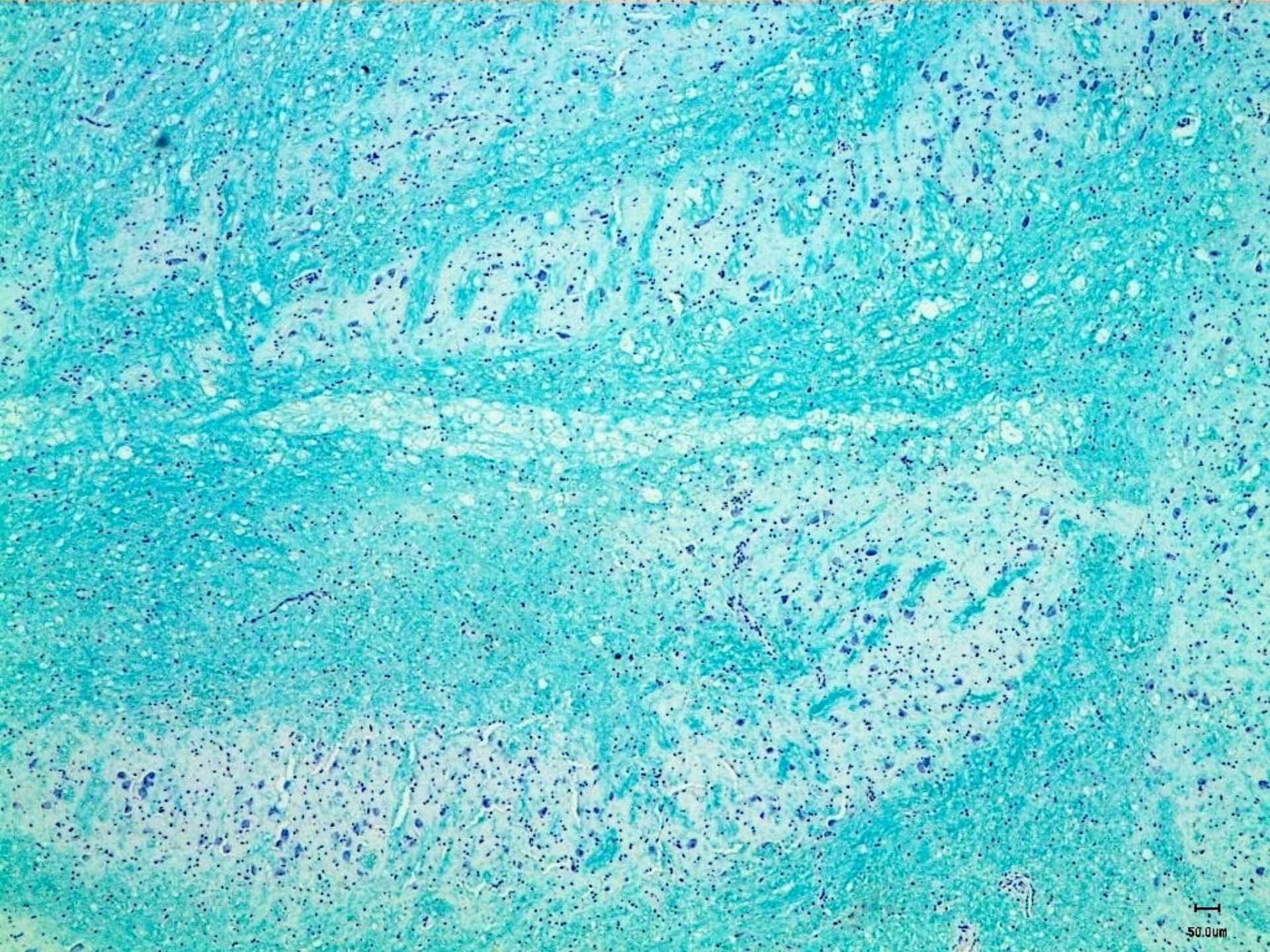


Mazzariol *et al.*, 2013



Osterhaus *et al.*, 1997





50.0um





UNKNOWN: VIRUS ENTRY



Available online at www.sciencedirect.com



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Comparative Immunology, Microbiology
and Infectious Diseases 33 (2010) 227–241

COMPARATIVE
IMMUNOLOGY
MICROBIOLOGY &
INFECTIOUS
DISEASES

www.elsevier.com/locate/cimid

Host–virus specificity of morbilliviruses predicted by structural modeling of the marine mammal SLAM, a receptor

Kazuo Ohishi^{a,*}, Akiko Ando^{a,b}, Rintaro Suzuki^c,
Kiyotaka Takishita^a, Masaru Kawato^a, Etsuko Katsumata^d,
Dai Ohtsu^e, Kenji Okutsu^c, Koji Tokutake^c,
Hirotaka Miyahara^f, Hirotaka Nakamura^g,
Tsukasa Murayama^b, Tadashi Maruyama^a

^a Japan Agency for Marine–Earth Science and Technology (JAMSTEC), Yokosuka,
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^f Okinawa Churaumi Aquarium, Motobu, Okinawa 905-0206, Japan

^g Kanagawa Zoo, Yokohama, Kanagawa 226-0042, Japan

Accepted 4 October 2008

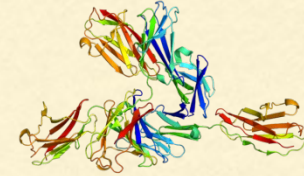
a.a. No.	Seal	Dog	Dolphin	Cow	Sheep	Human	Manatee
58	K	K	K	K	K	K	K
60	I	I	I	I	I	I	I
61	H	H	H	H	H	H	R*
63	L	L	L	L	L	V	V
65	T	T	T	T	T	T	T
67	A	A	A	A	A	A	E*
68	E	E	G*	E	E	K*	T*
69	S	S	S	S	S	S	S
72	N	N	D	D	D	N	S
73	S	S	T	T	T	S	T
74	V	I	V	V	V	V	F
75	K	K	K	K	K	E*	K
76	K	K	K	K	K	N*	K
77	K	K	K	K	K	K	K
80	S	S	S	S	S	S	S
82	D	D	D	D	D	D	D
84	P	P	R	R	R	S	S
85	E	E	K	K	K	E	E
87	G	G	D	D	G	G	G
90	R	R	H	R	H	R	P*
92	L	L	L	L	L	L	L
117	W	W	W	W	W	W	W
119	F	F	F	F	F	L	F
121	T	T	S	S	S	T	T
123	E	E	E	E	E	E	E
125	N	N	N	N	N	N	N
126	F	F	I	V	V	V	F
127	S	S	S	S	S	S	S
128	V	V	V	V	V	V	V
129	R*	Q	Q	Q	Q	Q	Q
130	H	H	Q	H	H	R	Q
131	F	F	F	F	F	F	F
Viruses	PDV, CDV	CDV	CMV	RPV, PPRV	PPRV, RPV	MV	None

The residue position, which varies among animals, is shaded. The light- and dark-shaded boxes indicate a variation in chemically (charge, hydrophilicity, etc.) similar or different residues. The asterisk indicates the specific residue with a chemical change for the animal SLAM. See the legend of Figure 2 for the animal names and accession numbers of the amino acid sequences used in this table.

Table 2. Amino acid residues on the SLAM interface possibly involved in regulating the binding and specificity of morbilliviruses.

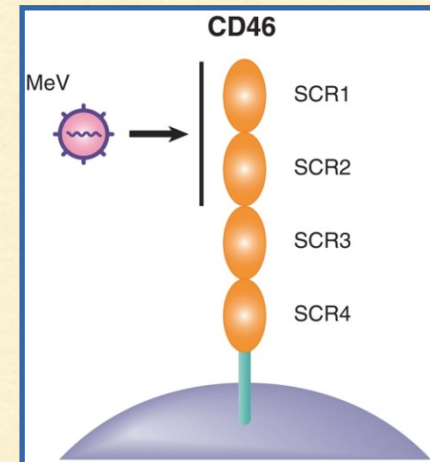
Basigin (CD147) - extracellular matrix metalloprotease (MMP) inducer

- . epithelial cells, neuronal cells, microglia (?)
- . endothelial cells, macrophages
- . leukocytes



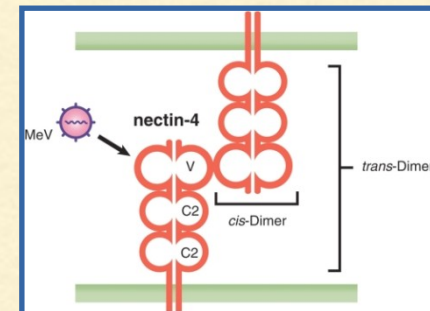
Complement regulatory protein (CD46)

- . All nucleated cells
- . not used by CDV and RPV



Nectin IV

- . All nucleated cells (virus shedding)
- . not used by CDV and RPV



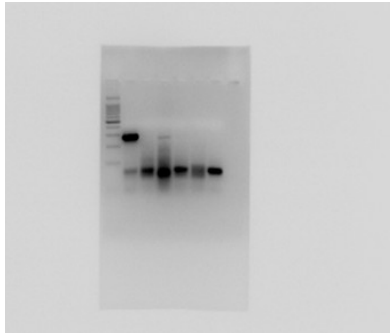
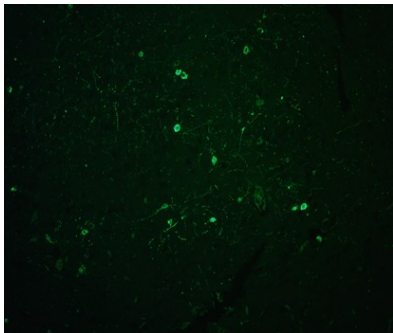
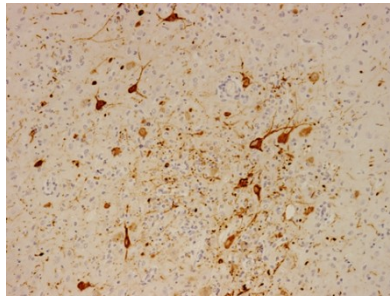
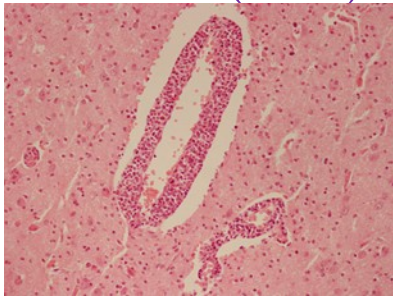
GAG; pro-HB-EGF (PDV, Vero cells)

Infezione da DMV in *S. coeruleoalba*

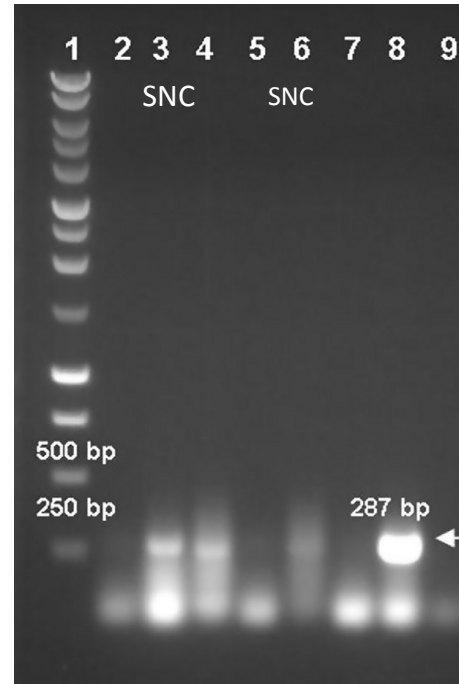
“Brain-only” forms of DMV infection

(Domingo *et al.*, 1995; Soto *et al.*, 2011)

Un nuovo “modello di studio” nei confronti di SSPE (uomo) e di ODE (cane)???

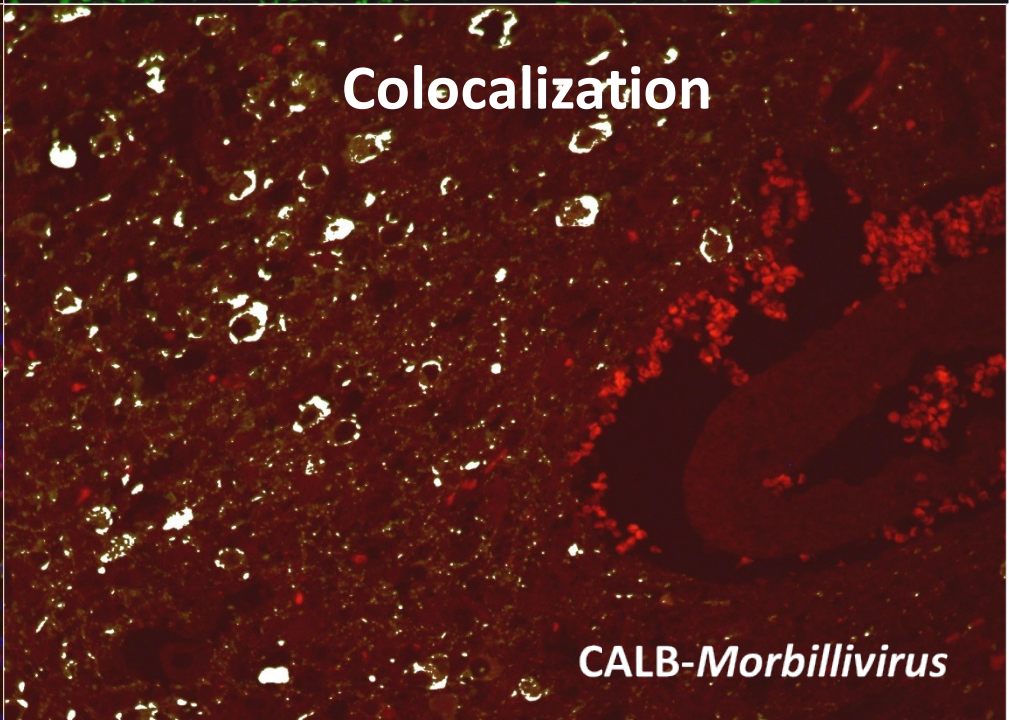
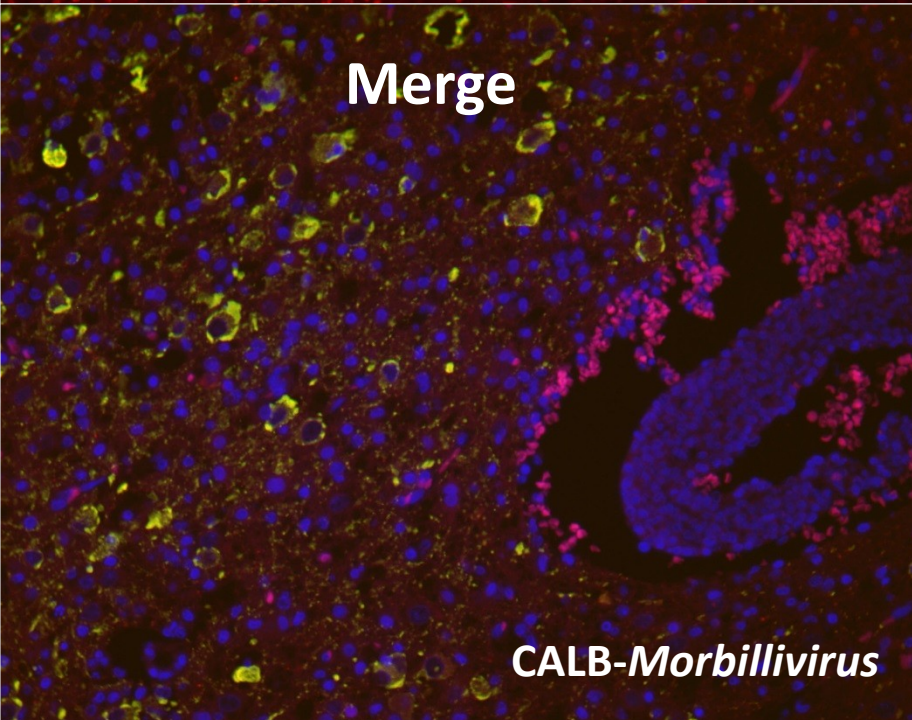
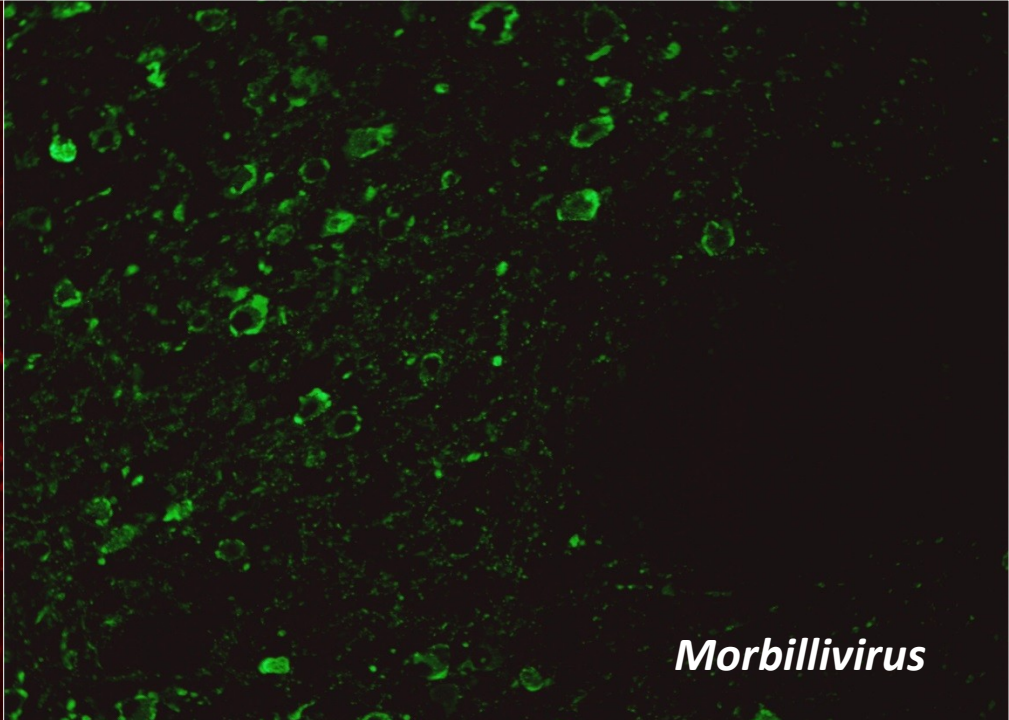
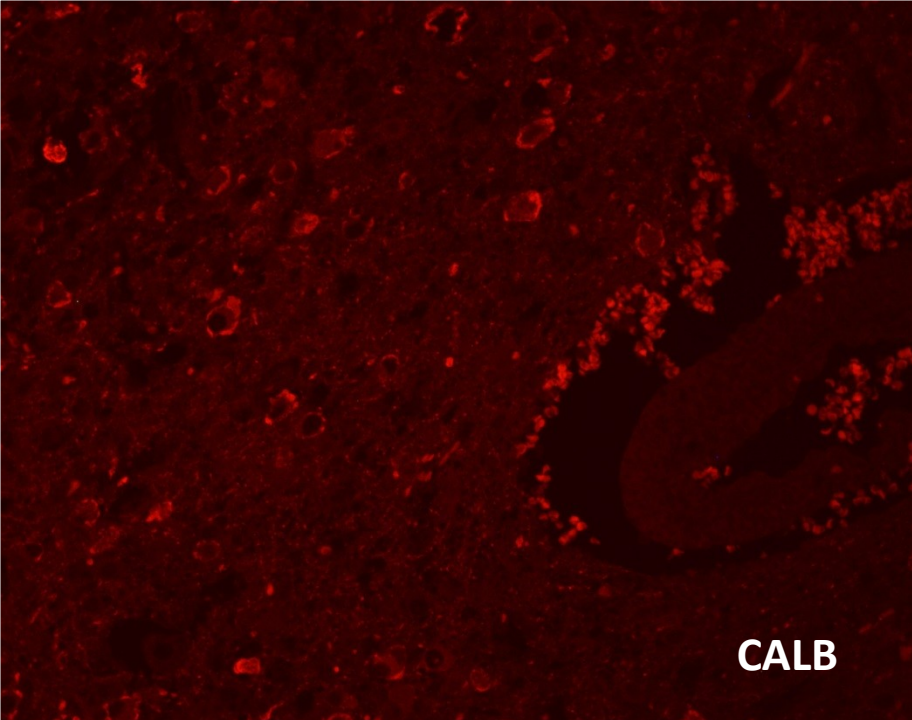


(Di Guardo *et al.*, 2011; Di Guardo & Mazzariol, 2016)



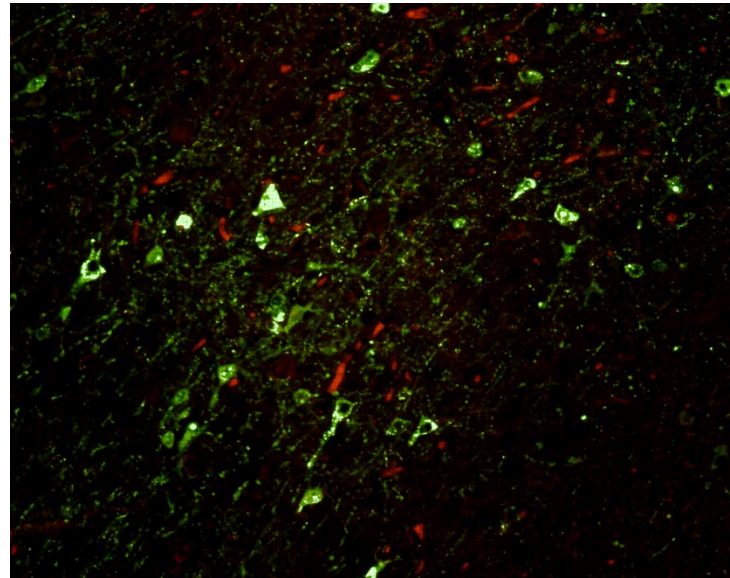
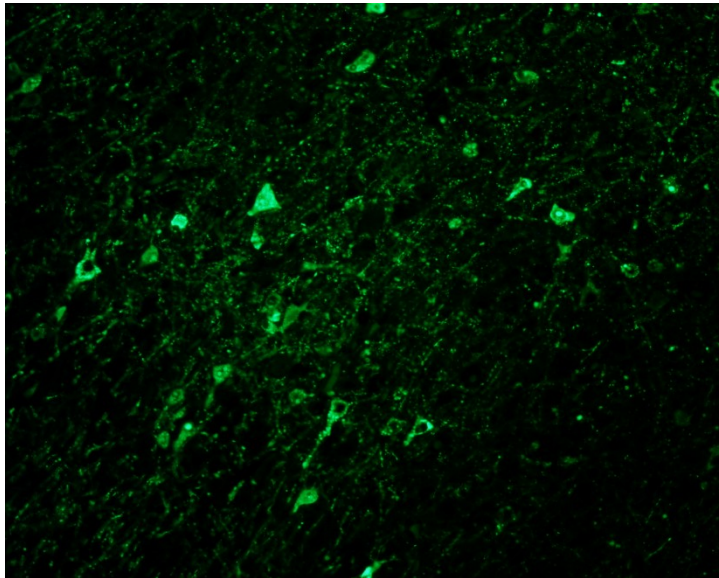
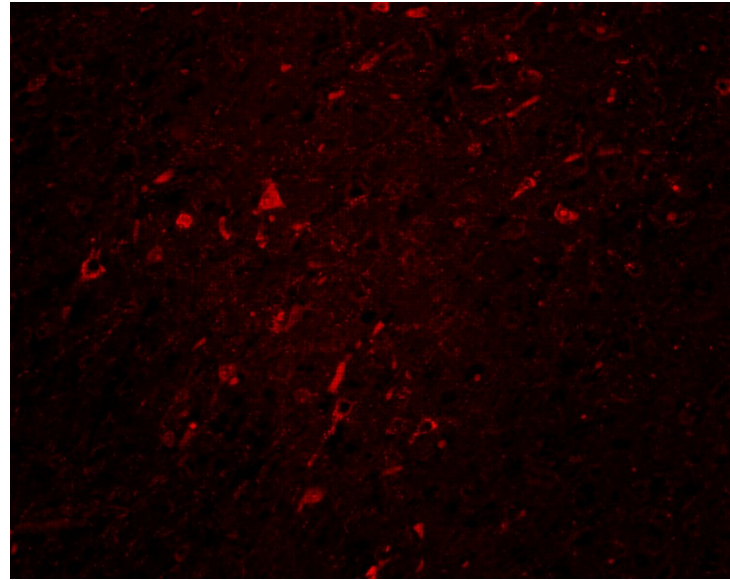
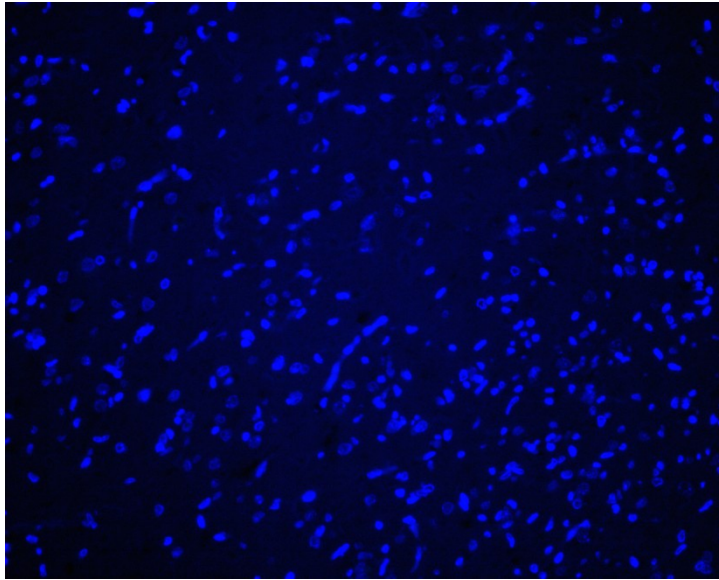
Analoga positività in *T. truncatus*

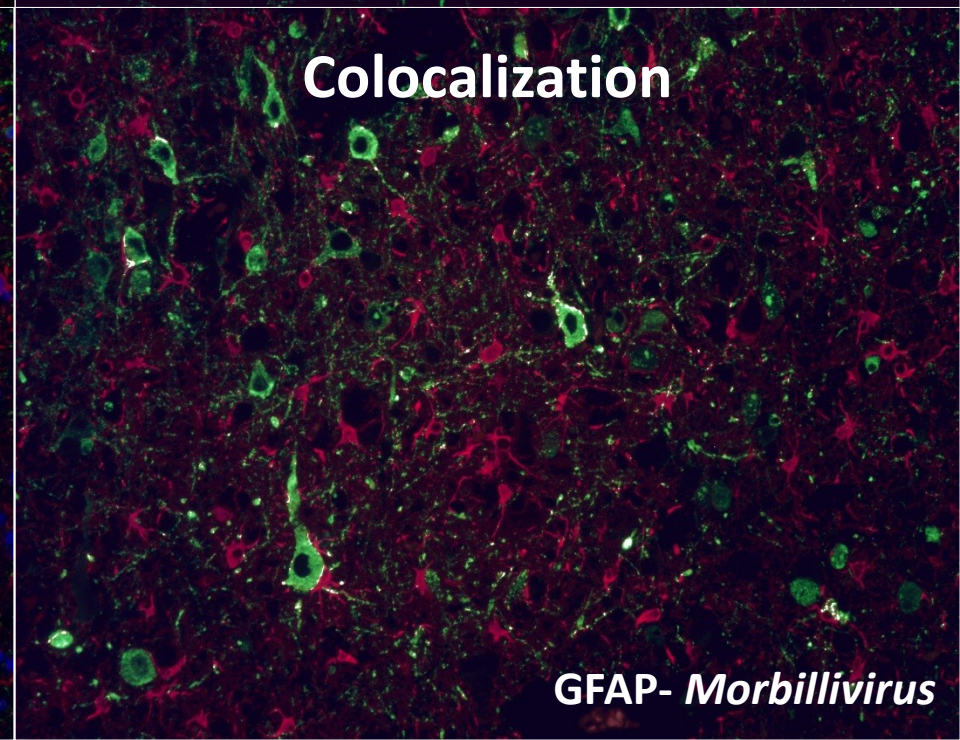
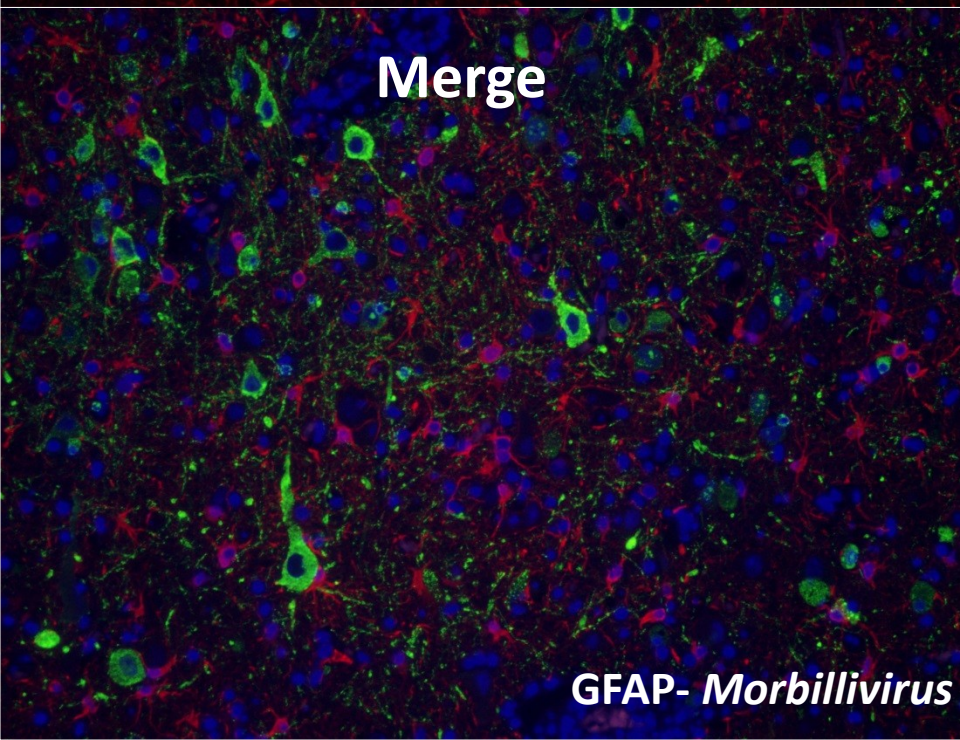
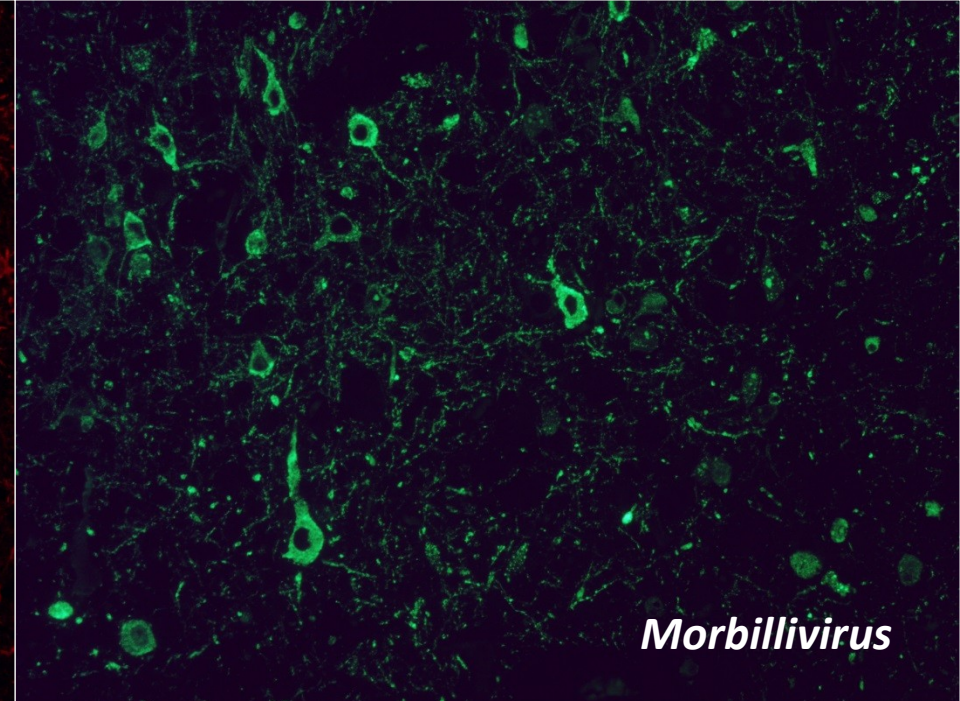
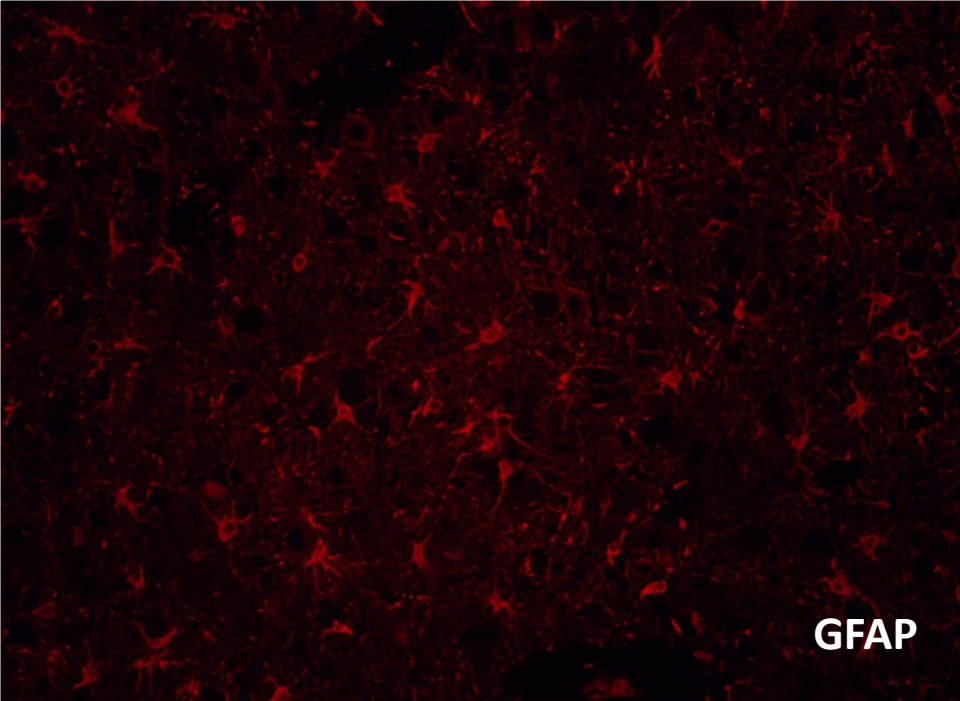
(Di Guardo *et al.*, 2013)

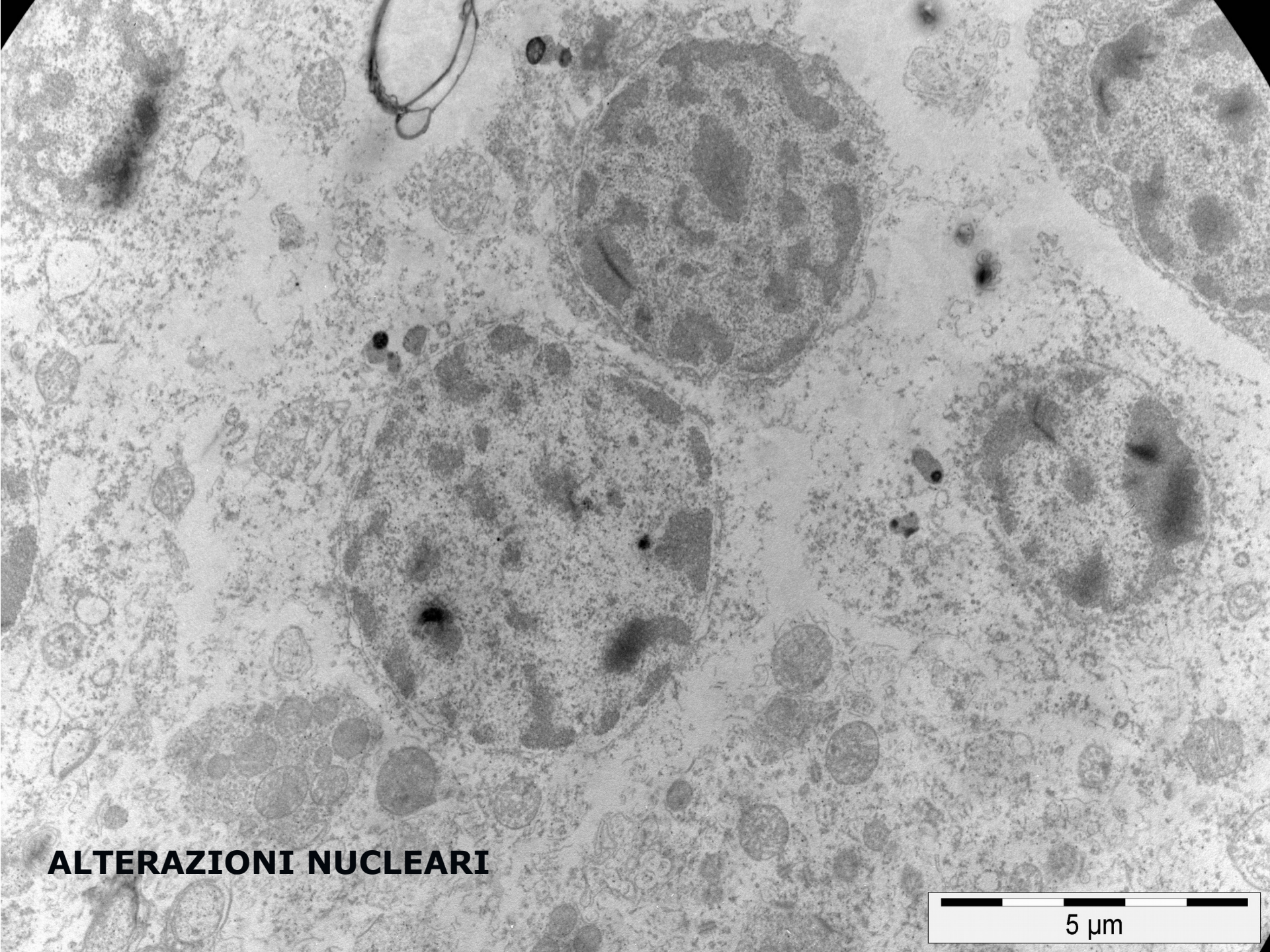


nNOS-IR

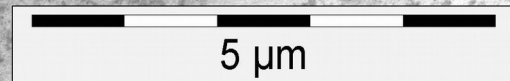
Immagine (20X): in senso orario, DAPI, nNOS, *Morbillivirus*, Colocalizzazione.

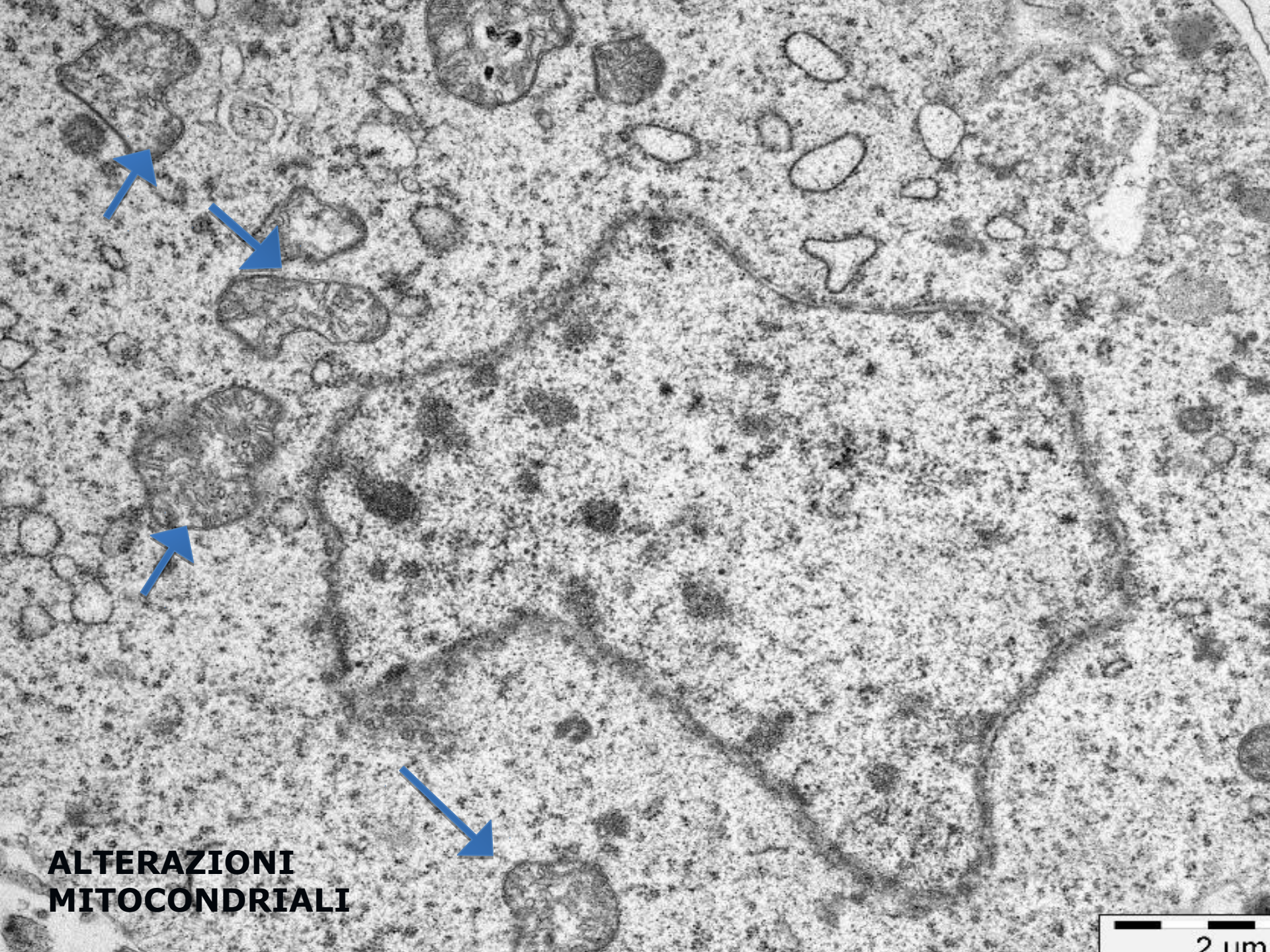






ALTERAZIONI NUCLEARI

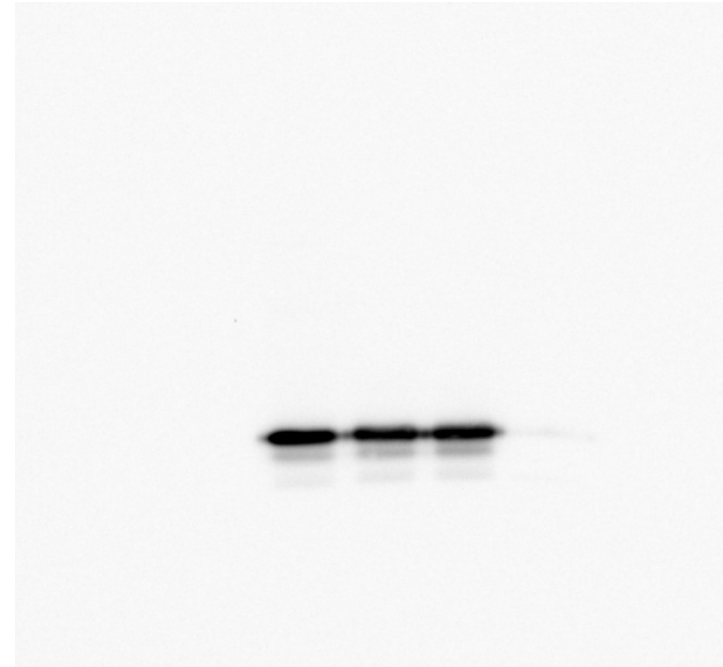
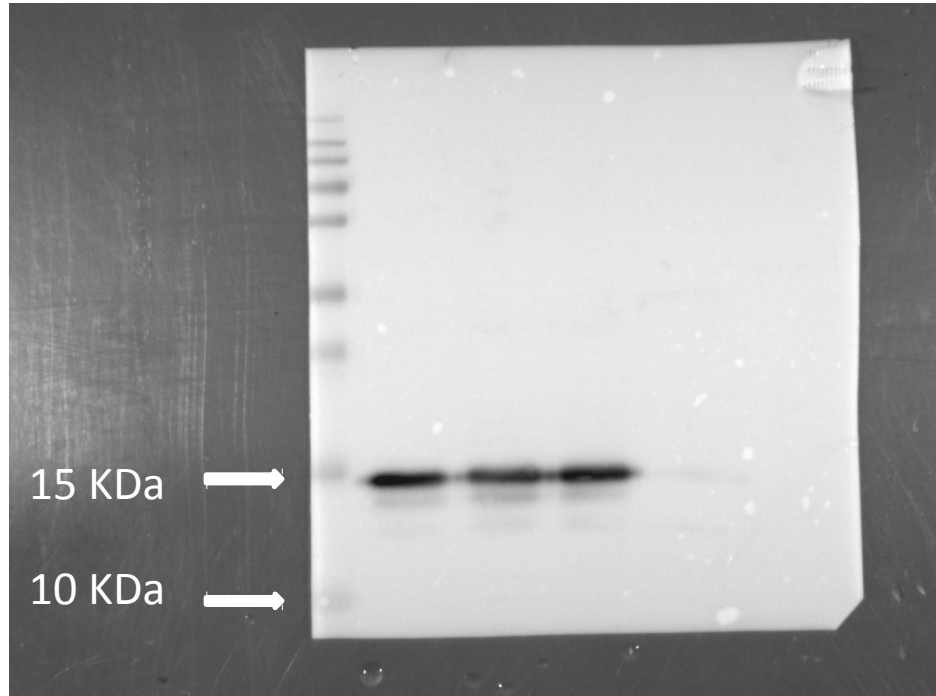




**ALTERAZIONI
MITOCONDRIALI**

2 μ m

LC3 (autophagy biomarker)

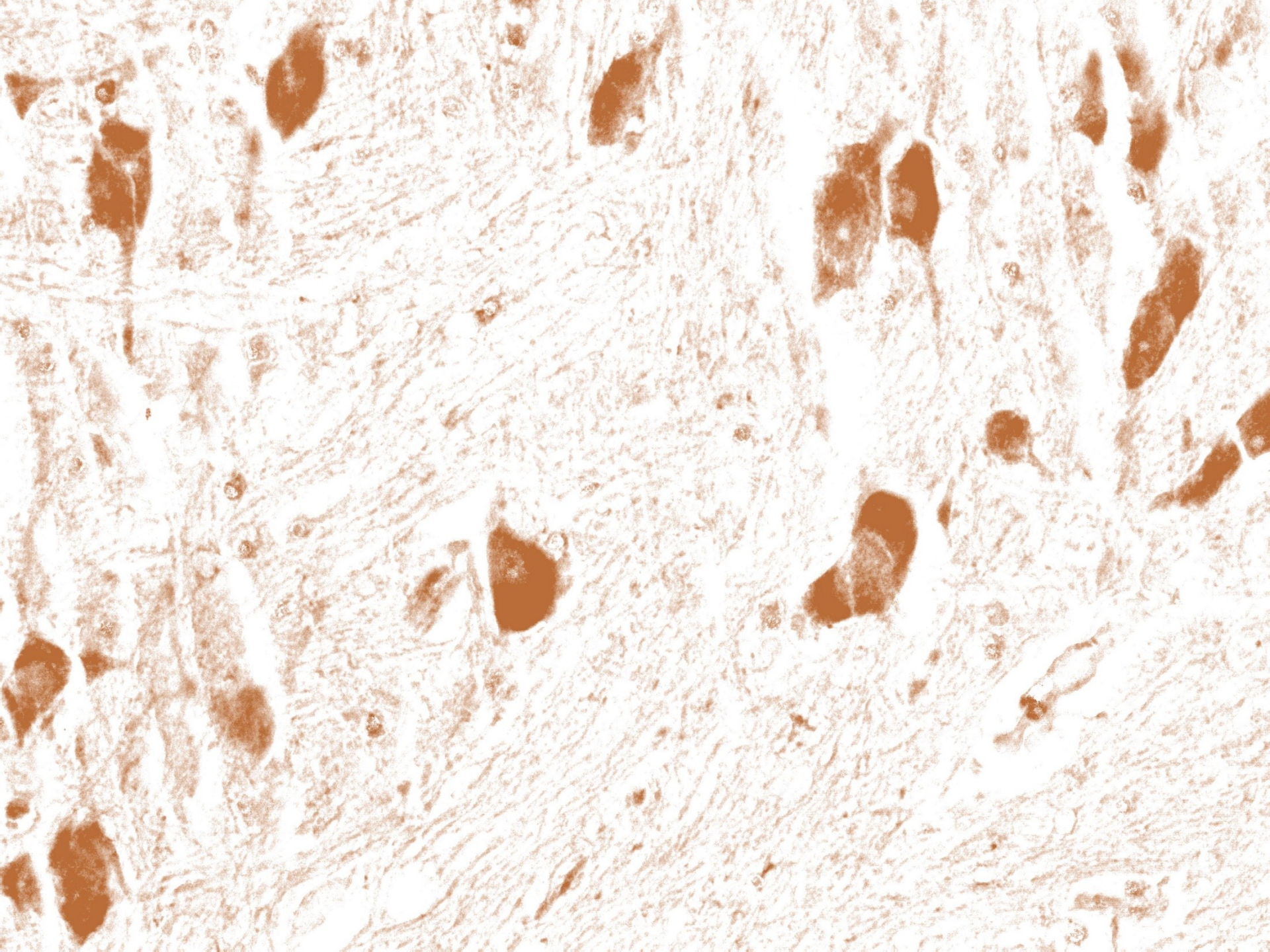


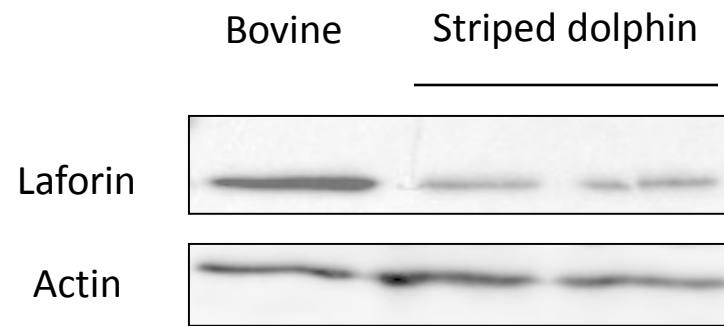
Lane 1: Molecular weight (MW) ladder/marker

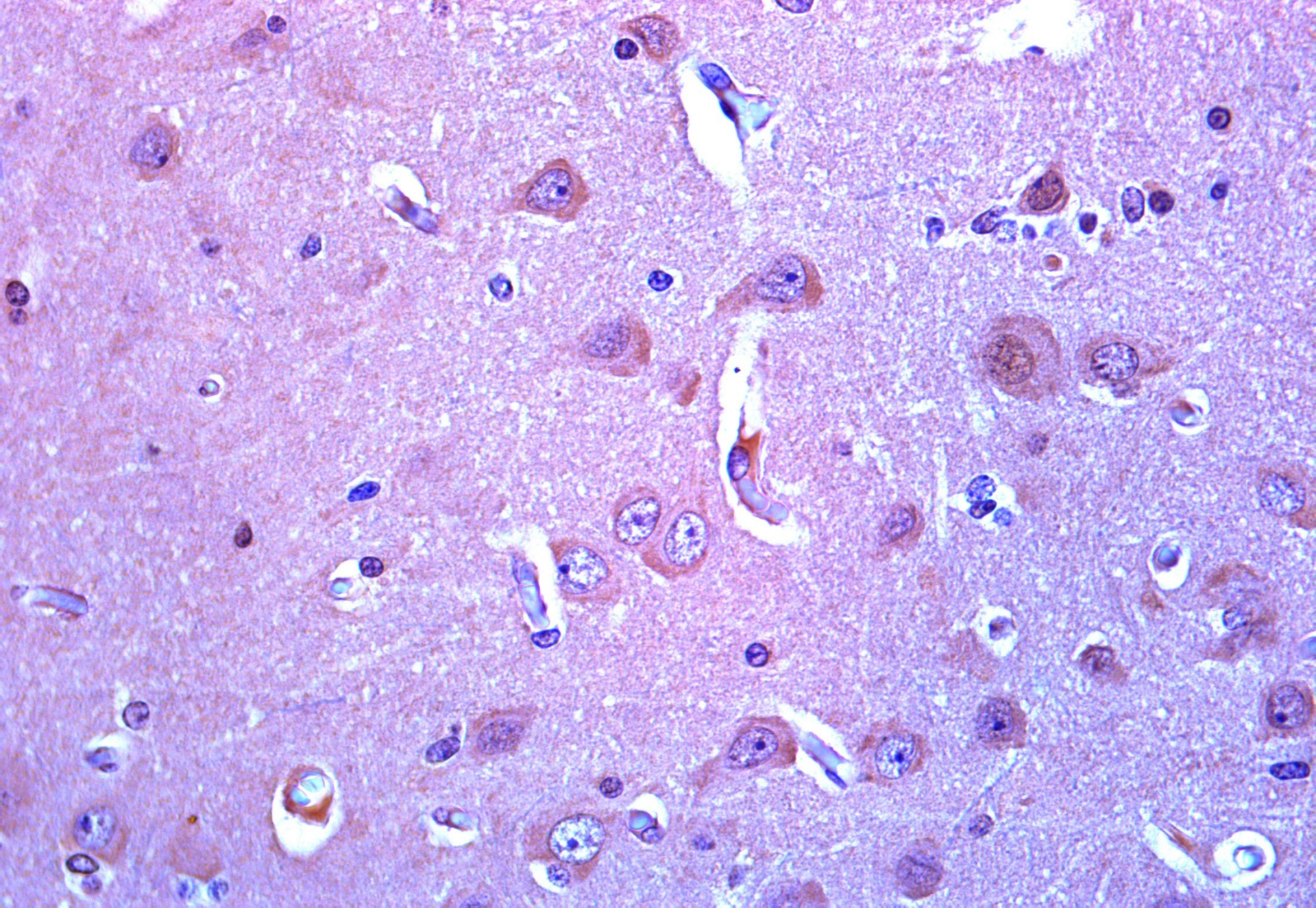
Lane 2-4: Brain tissue homogenates from three (3) striped dolphins (*Stenella coeruleoalba*)

Lane 5: THP-1

MW of human LC3 = 17 kDa (approximately)







Striped dolphin (*Stenella coeruleoalba*). Brain (*hippocampus*). Laforin IHC staining (by means of an anti-laforin/EPM2A primary antibody, diluted 1:50). 40X ob., Mayer's haematoxylin counterstain.

Considerazioni conclusive - 1

- Evidenza d'infezione da *Morbillivirus*, *T. gondii* e *B. ceti* nei cetacei dei nostri mari.
- Presenza e circolazione “datate” di *Morbillivirus* e *T. gondii* fra i cetacei dei nostri mari.
- Recente “ingresso” con presenza “sporadica” dell'infezione da *B. ceti* nelle popolazioni di *S. coeruleoalba* del Mar Mediterraneo ed, in particolare, in quelle dei mari italiani.
- Rilevanza delle indagini sierologiche per la sorveglianza epidemiologica delle infezioni da *Morbillivirus*, *T. gondii* e *Brucella* spp. nei cetacei spiaggiati (anche in virtù della documentata capacità zoonosica di *T. gondii* e *Brucella* spp.).



Considerazioni conclusive - 2

- Valenza strategica ed utilità di una “Banca Nazionale dei Sieri dei Cetacei Spiaggiati Lungo le Coste Italiane”.
- Nuove acquisizioni sulla neuropatogenesi dell'infezione da Morbillivirus (marcata colonizzazione dei neuroni **CALB-IR** con ridotta colonizzazione **astrocitaria** ad opera del virus) in *S. coeruleoalba* (“*brain-only*” forms of DMV infection).
- Importanza dello studio dei determinanti biologici “virus-specifici” (eventi mutazionali come nella **SSPE???**) ed “ospite-specifici” (recettori virali, immunità, autofagia) responsabili della reiterata persistenza di DMV nel tessuto cerebrale di *S. coeruleoalba* e di *T. truncatus*, con successivo sviluppo di “brain-only” forms of DMV infection.
- Importanza dello studio delle dinamiche d'interazione “DMV-contaminanti ambientali immunotossici e neurotossici” (rapporti eziopatogenetici fra **PrPc** e *B. ceti*?).



RINGRAZIAMENTI

*Ministero dell'Ambiente
e della Tutela del
Territorio e del Mare*

Ministero della Salute

*Rete Nazionale per la Sorveglianza
Sanitaria sui Cetacei Spiaggiati lungo le
Coste Italiane (Università, II.ZZ.SS.,
C.Re.Di.Ma., Banca Tessuti Mammiferi
Marini del Mediterraneo, I.S.P.R.A., I.S.S.
e tutte le altre Istituzioni ed Enti coinvolti)*





*Il mio unico desiderio è
diventare il fiore del cactus
campanulus che a quest'ora
si sarà già richiuso.
ma dovrei aspettare
una nuova fioritura.*

Marco Pedone

n.11.5.1958

m.27.8.2009

A photograph of a dolphin leaping from the surface of the ocean. The dolphin is captured mid-air, with its body arched and its tail still partially submerged, creating a splash of white water. The ocean is a deep blue with visible ripples. In the upper right corner, there is a blue, cloud-like thought bubble with a black outline. Inside the bubble, the text "THANKS SO MUCH FOR YOUR KIND ATTENTION!!!" is written in a bold, yellow, sans-serif font. Three smaller blue circles of increasing size lead from the bottom of the main bubble towards the dolphin's head.

**THANKS SO
MUCH FOR
YOUR KIND
ATTENTION!!!**