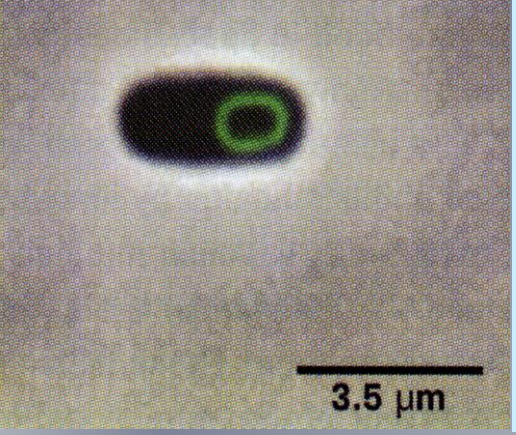

Carrera de Especialización en Esterilización

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Asignatura: Seguridad Operativa

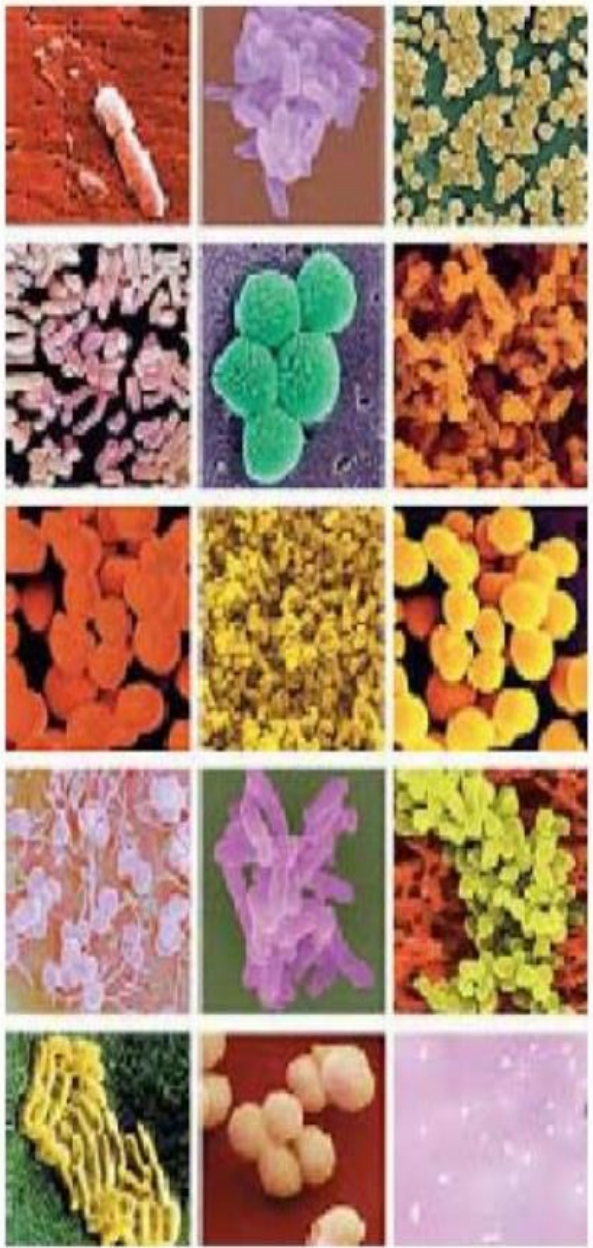
Clase 6

8 de Junio de 2017

Universidad Nacional de Rosario

Consejo Nacional de Investigaciones

MICROBIOLOGÍA



"La ciencia encargada del estudio de los microorganismos tan pequeños cuya visualización requiere del microscopio“ abarca:

partículas no celulares como los virus, viroides y priones, hasta organismos celulares tan diferentes como las bacterias, los protozoos y parte de las algas y de los hongos.

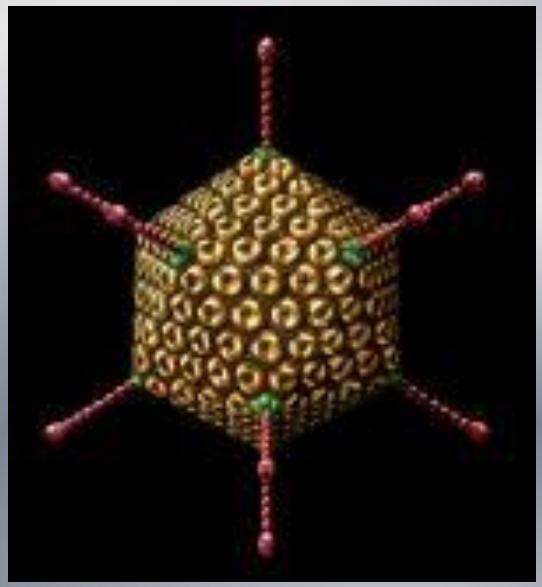
Viruses, Viroids, and Prions

Are Viruses Living or Non-living?

- Viruses are both and neither
- They have some properties of life but not others
- For example, viruses can be killed, even crystallized like table salt

What are Viruses?

- A virus is a non-cellular particle made up of genetic material and protein that can invade living cells.



Viral History

Discovery of Viruses

- Beijerinck (1897) coined the Latin name "virus" meaning poison
- He studied filtered plant juices & found they caused healthy plants to become sick

Tobacco Mosaic Virus

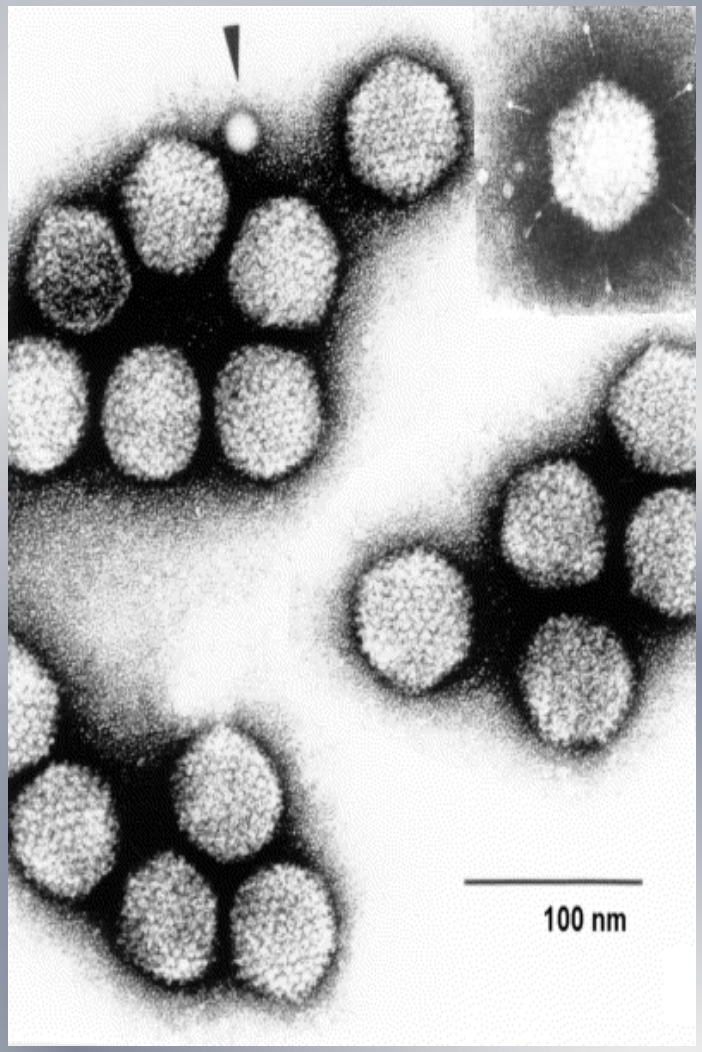
- Wendell Stanley (1935) crystallized sap from sick tobacco plants
- He discovered viruses were made of nucleic acid and protein

Smallpox

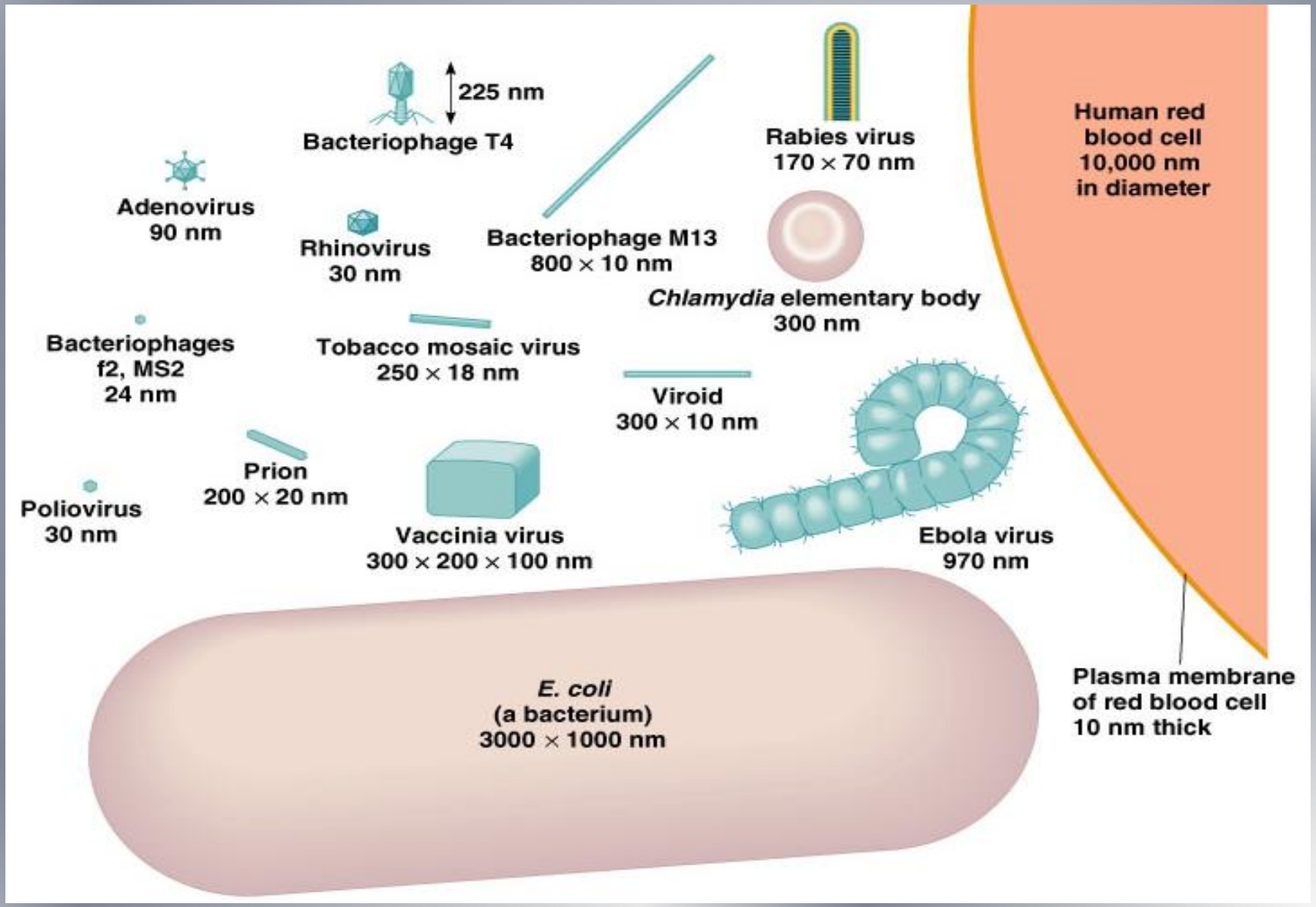
- Edward Jenner (1796) developed a smallpox vaccine using milder cowpox viruses
- Deadly viruses are said to be virulent
- Smallpox has been eradicated in the world today

Viewing Viruses

- Viruses are smaller than the smallest cell
- Measured in nanometers
- Viruses couldn't be seen until the electron microscope was invented in the 20th century



Size of Viruses



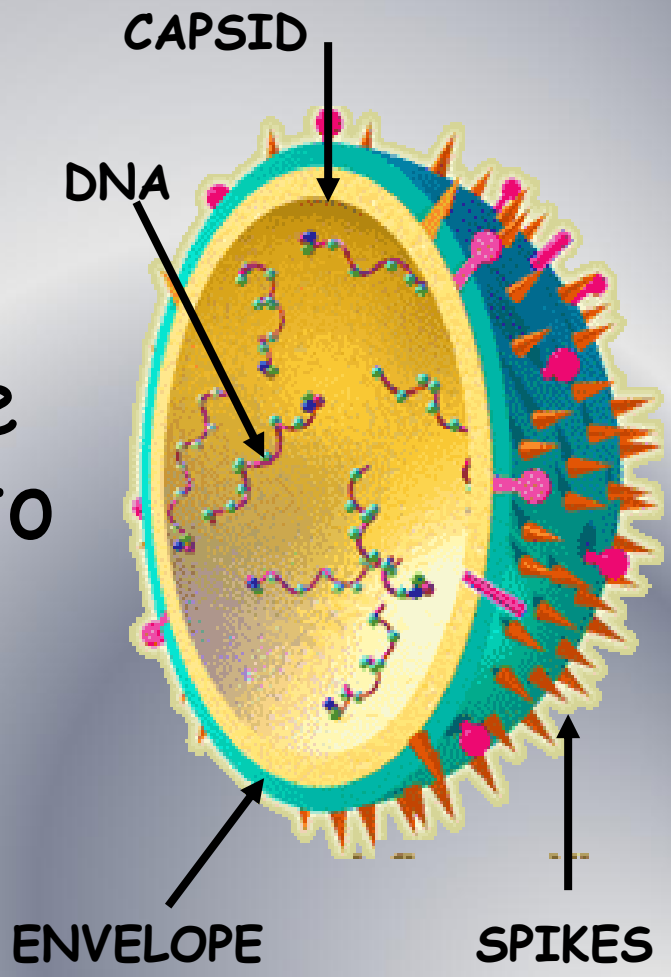
Viral Structure

Characteristics

- Non living structures
- Noncellular
- Contain a protein coat called the capsid
- Have a nucleic acid core containing DNA or RNA
- Capable of reproducing only when inside a HOST cell

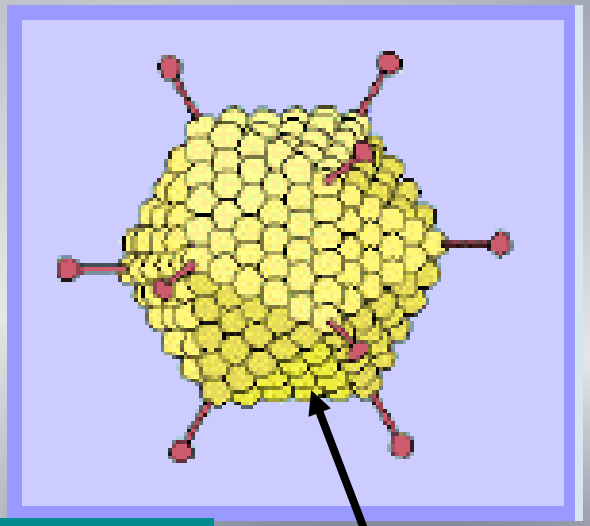
Characteristics

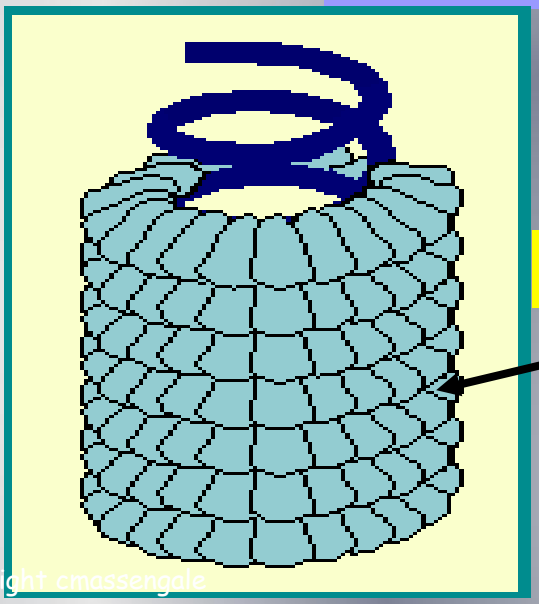
- Some viruses are enclosed in an protective envelope
- Some viruses may have spikes to help attach to the host cell
- Most viruses infect only SPECIFIC host cells



Characteristics

- Viral capsids (coats) are made of individual protein subunits
- Individual subunits are called capsomeres

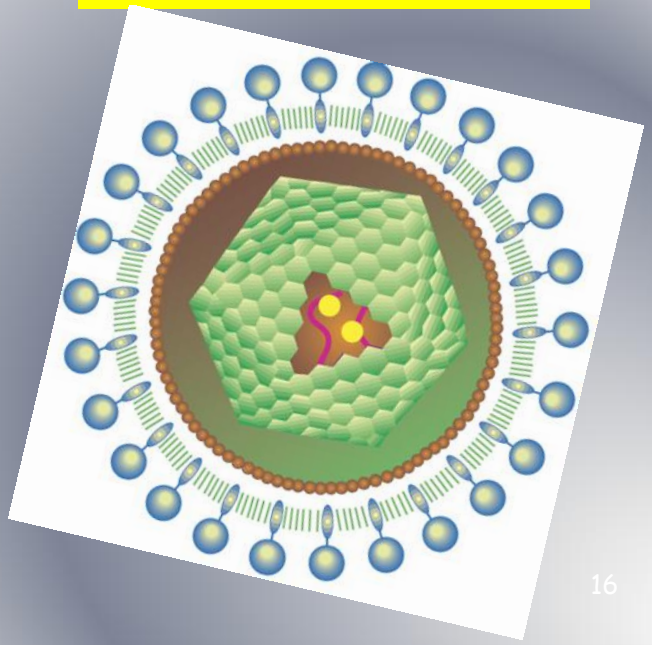




Characteristics

- Outside of host cells, viruses are inactive
- Lack ribosomes and enzymes needed for metabolism
- Use the raw materials and enzymes of the host cell to be able to reproduce

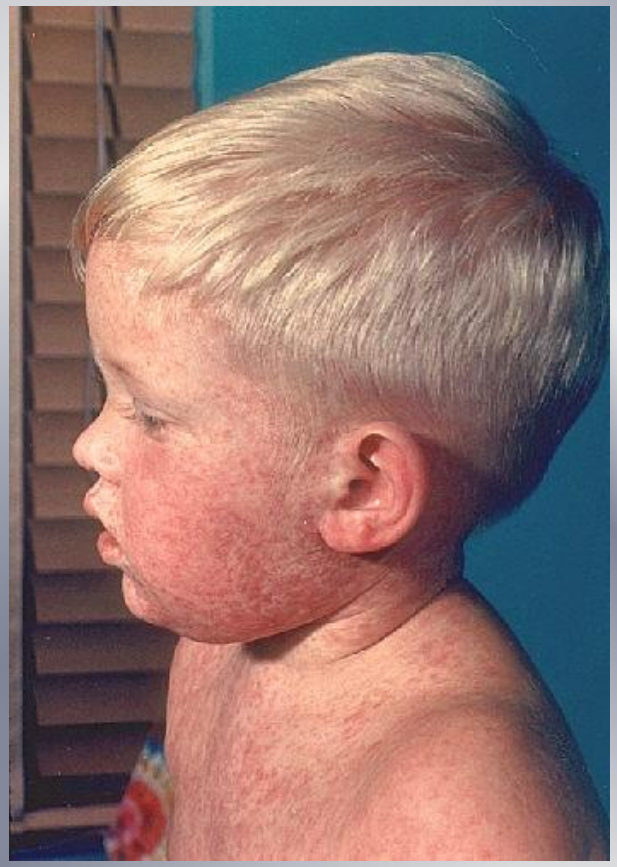
EBOLA VIRUS



HIV VIRUS

Characteristics

- Some viruses cause disease
- Smallpox, measles, mononucleosis, influenza, colds, warts, AIDS, Ebola
- Some viruses may cause some cancers like leukemia

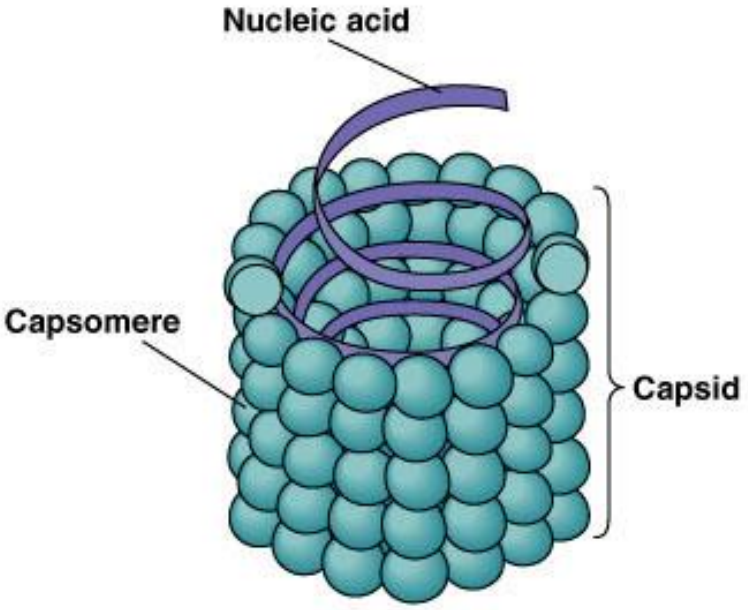


MEASLES

Viral Shapes

- Viruses come in a variety of shapes
- Some may be helical shape like the Ebola virus
- Some may be polyhedral shapes like the influenza virus
- Others have more complex shapes like bacteriophages

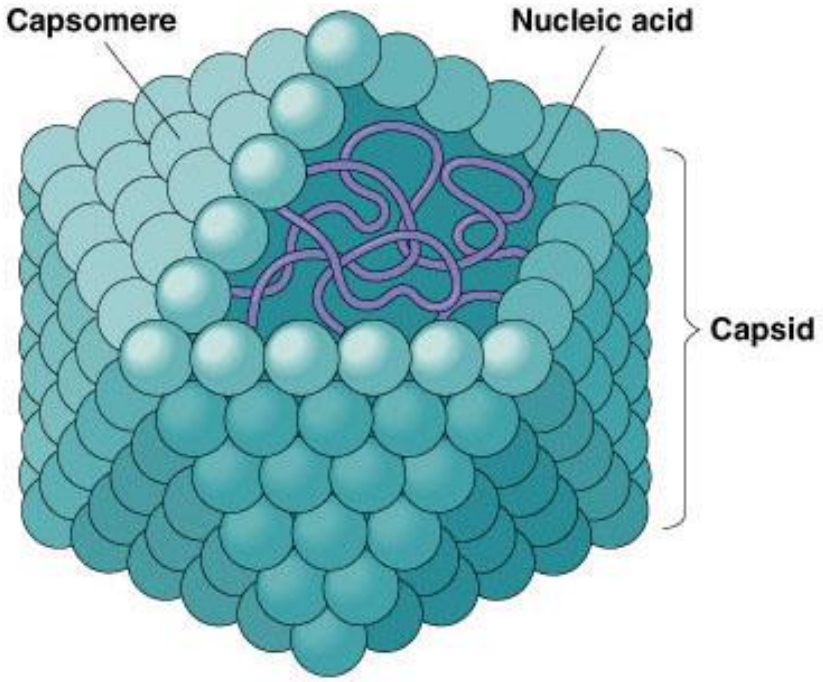
Helical Viruses



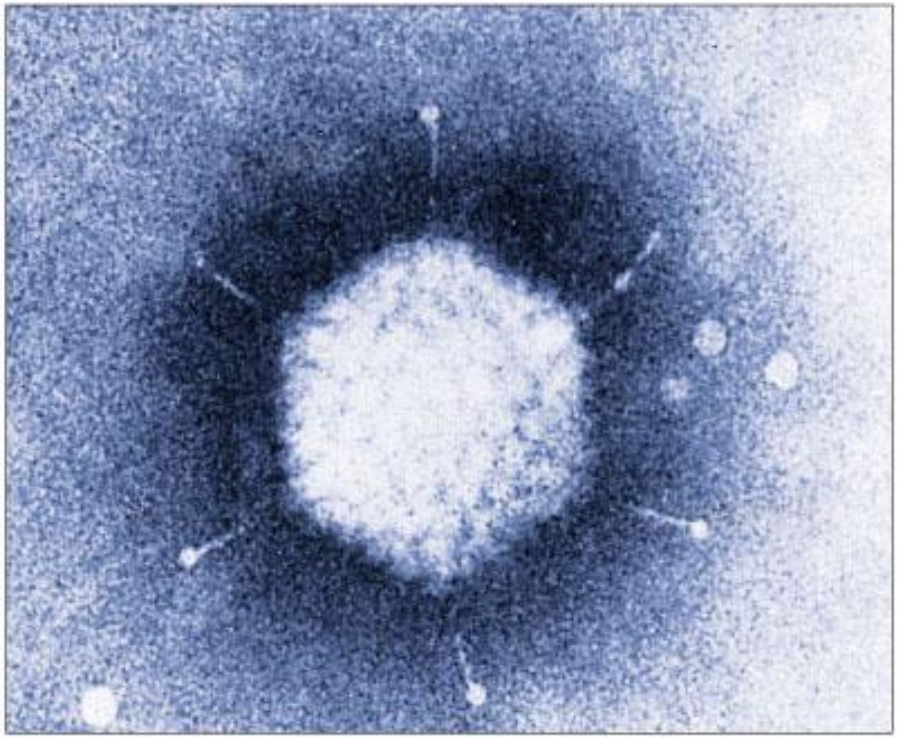
(a) A helical virus

(b) Ebola virus

Polyhedral Viruses

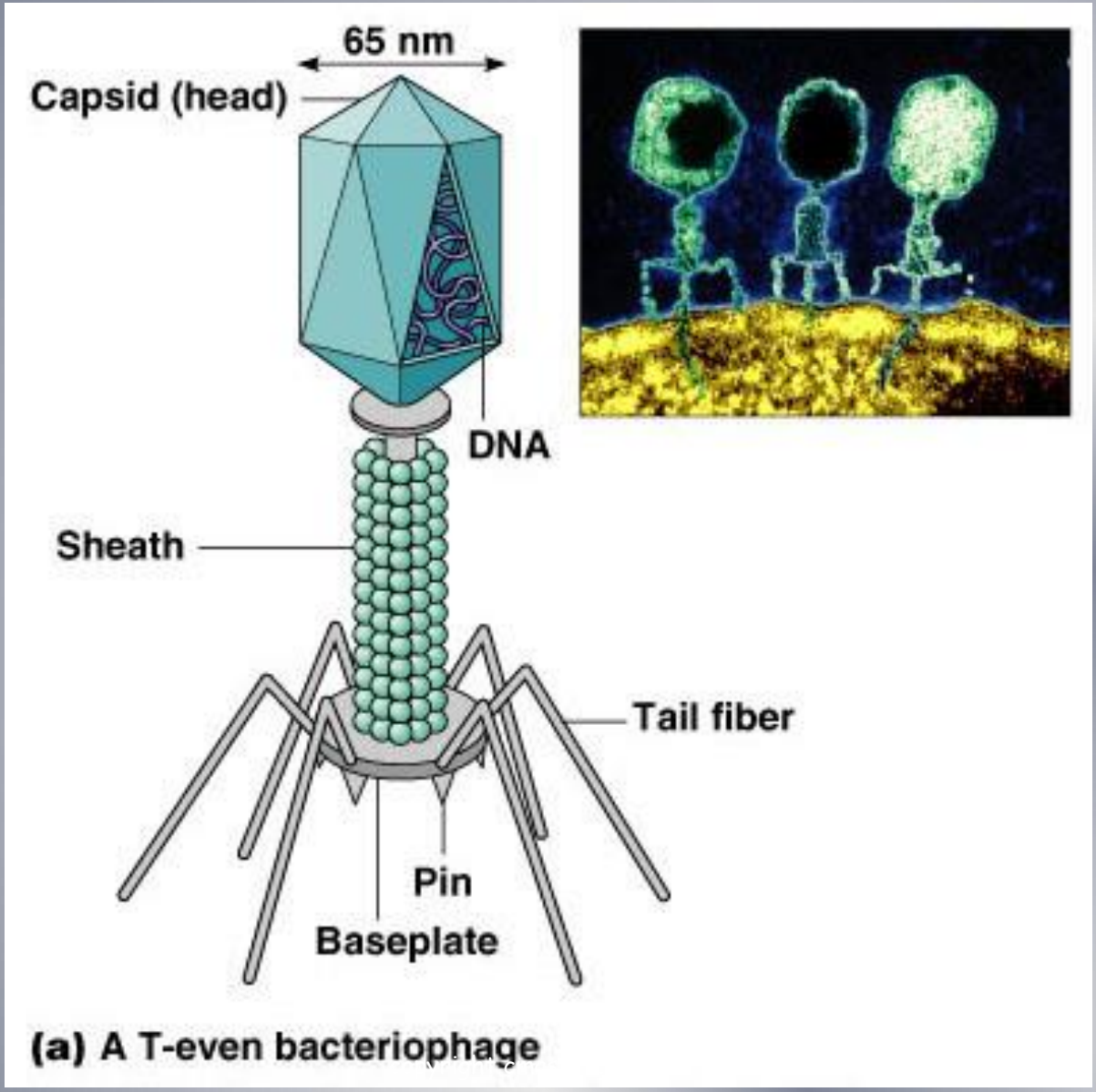


(a) A polyhedral virus



(b) A *Mastadenovirus*

Complex Viruses



Taxonomy of Viruses

Viral Taxonomy

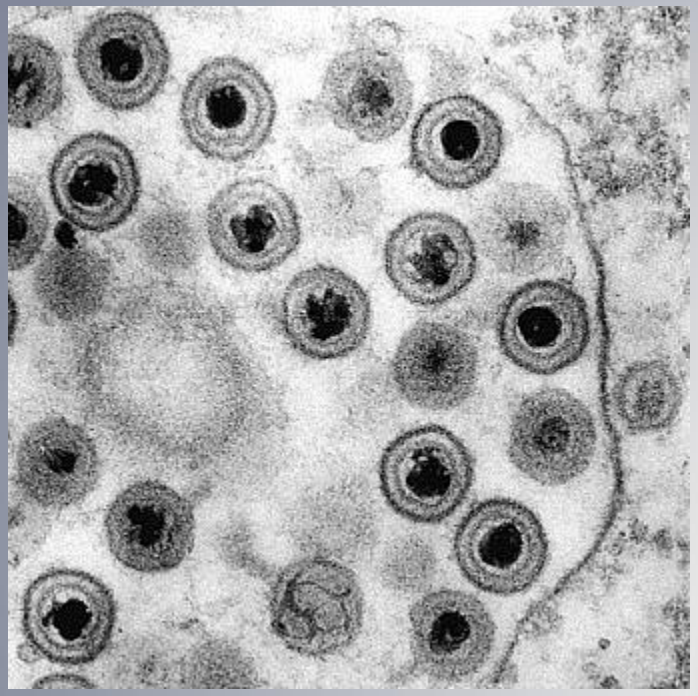
- Family names end in -viridae
- Genus names end in -virus
- Viral species: A group of viruses sharing the same genetic information and ecological niche (host).
- Common names are used for species
- Subspecies are designated by a number

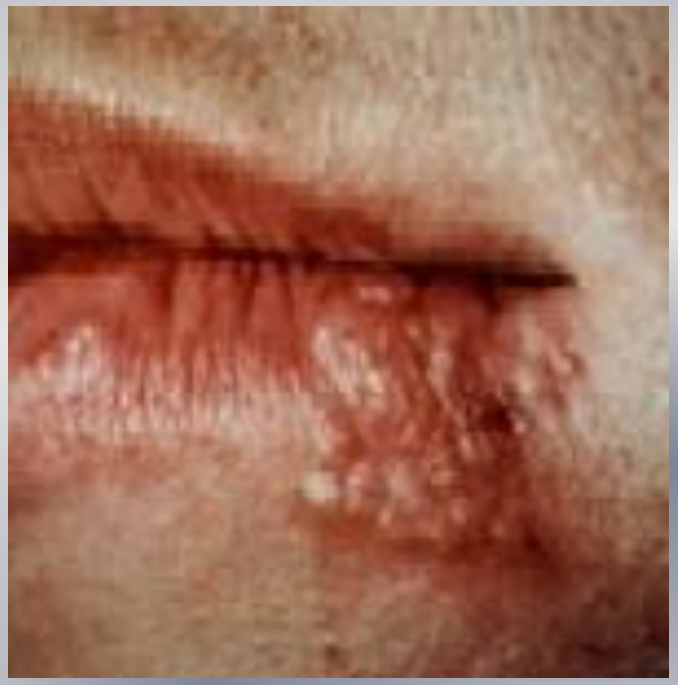
Viral Taxonomy Examples

- Herpesviridae
- Herpesvirus
- Human herpes virus 1, HHV 2, HHV 3

- Retroviridae
- Lentivirus
- Human Immunodeficiency Virus 1, HIV 2

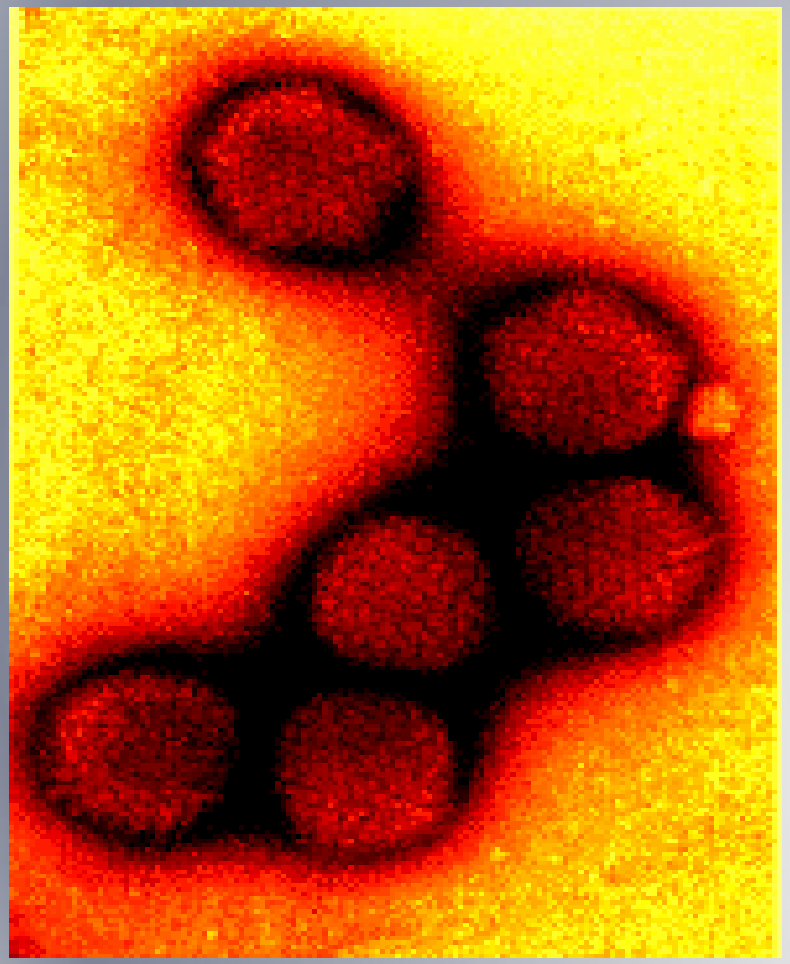
Herpes Virus

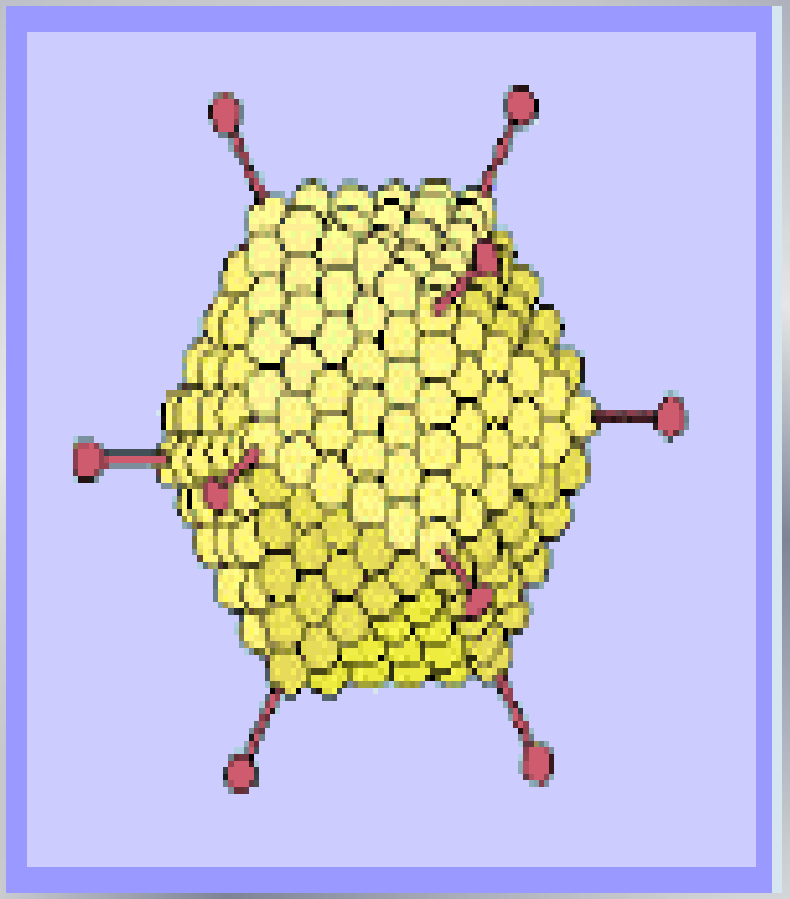




SIMPLEX I and II

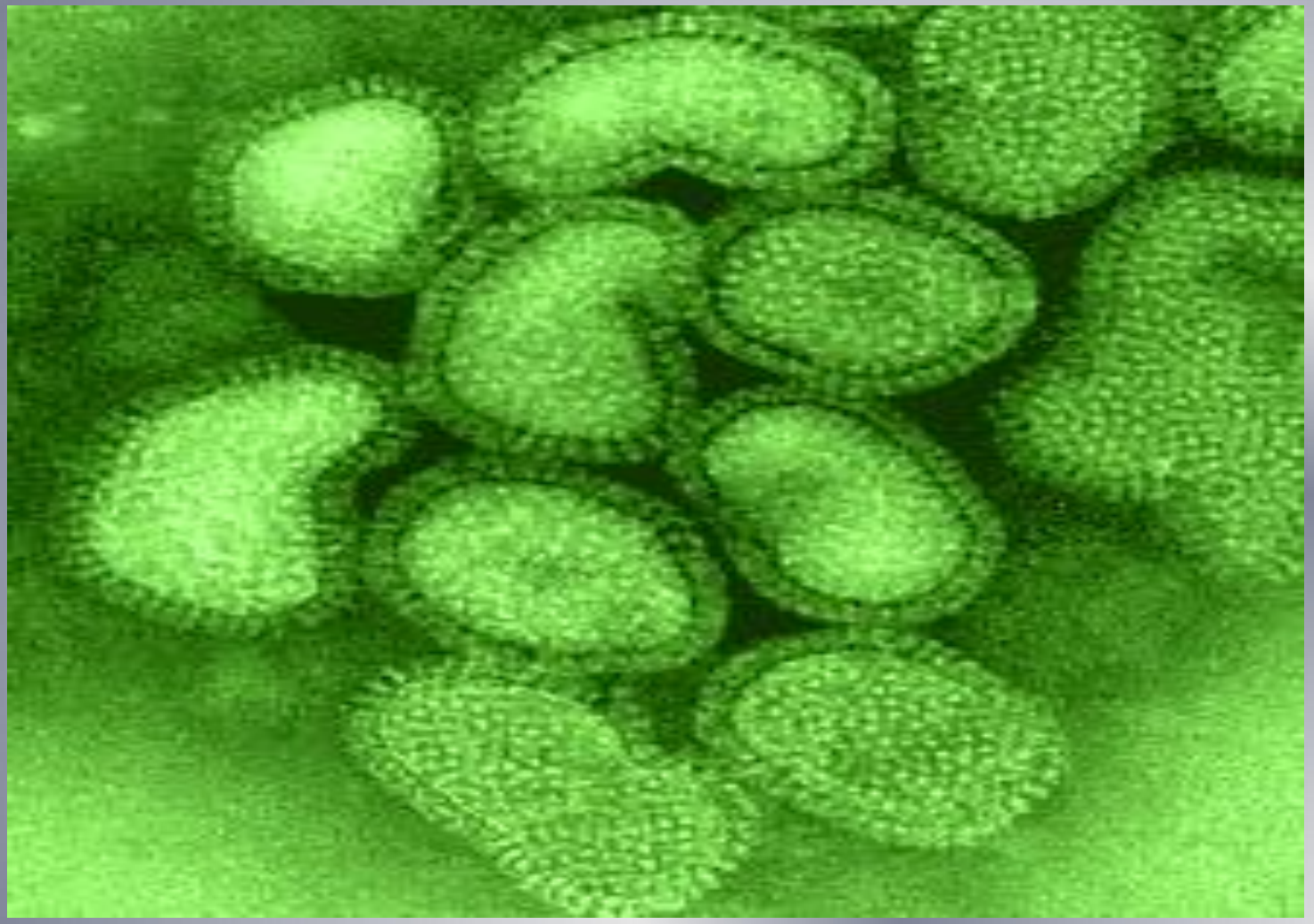
Adenovirus



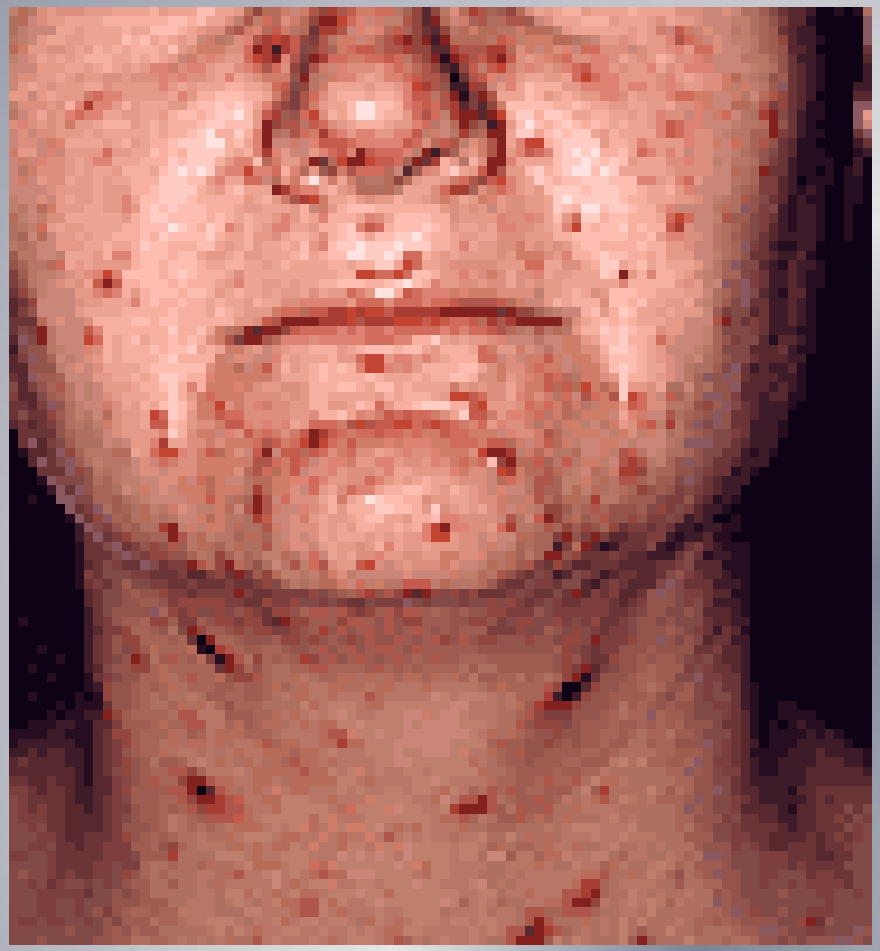


COMMON COLD

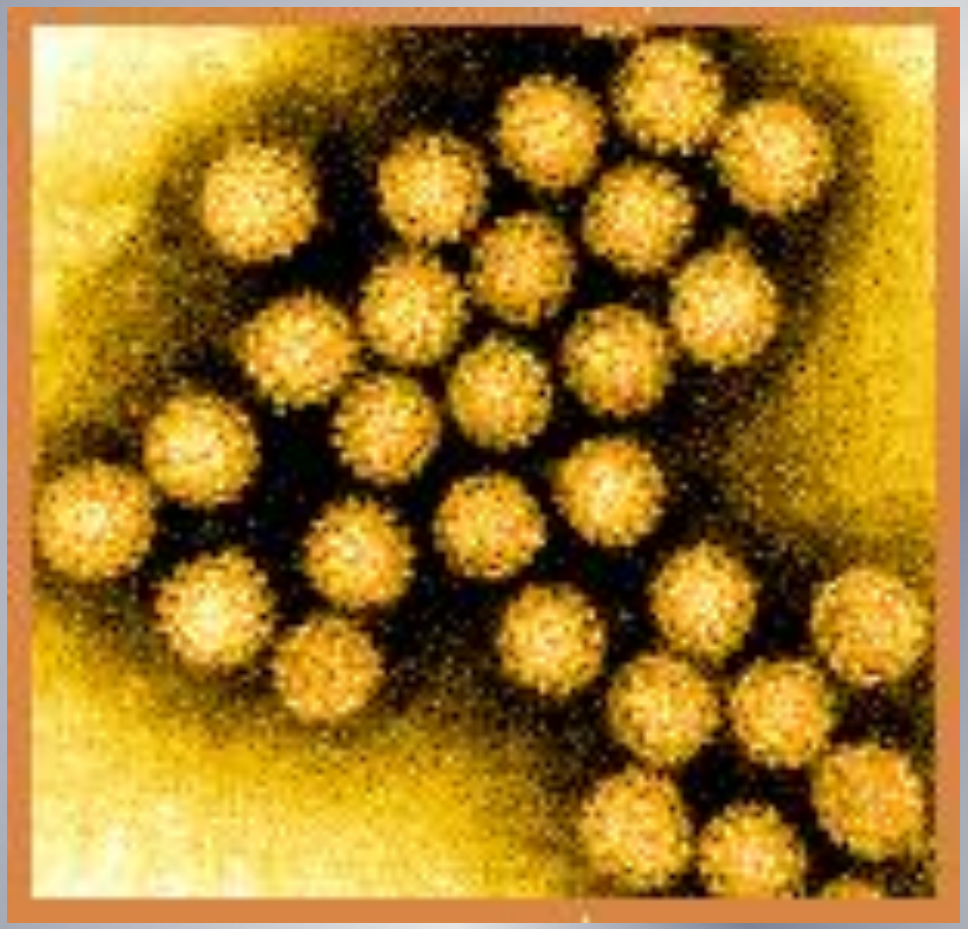
Influenza Virus



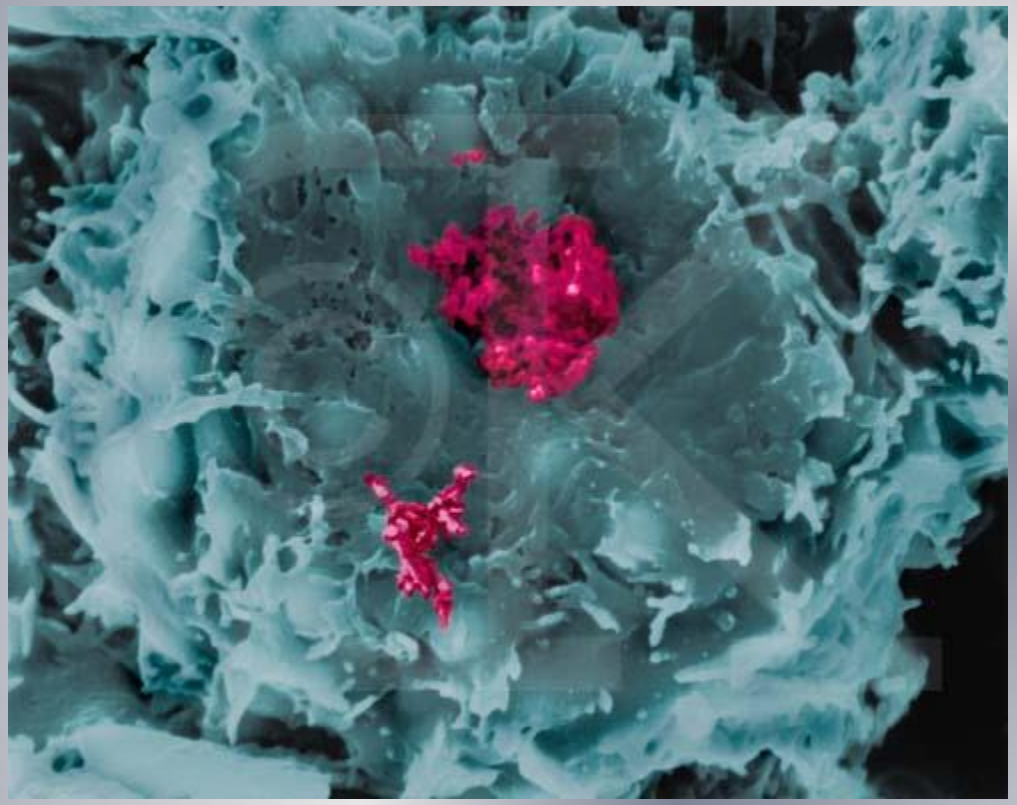
Chickenpox (Varicela) Virus



Papillomavirus – Warts!



Retroviruses



Characteristics of Retroviruses

- Contain RNA, not DNA
- Family Retroviridae
- Contain enzyme called Reverse Transcriptase
- When a retrovirus infects a cell, it injects its RNA and reverse transcriptase enzyme into the cytoplasm of that cell

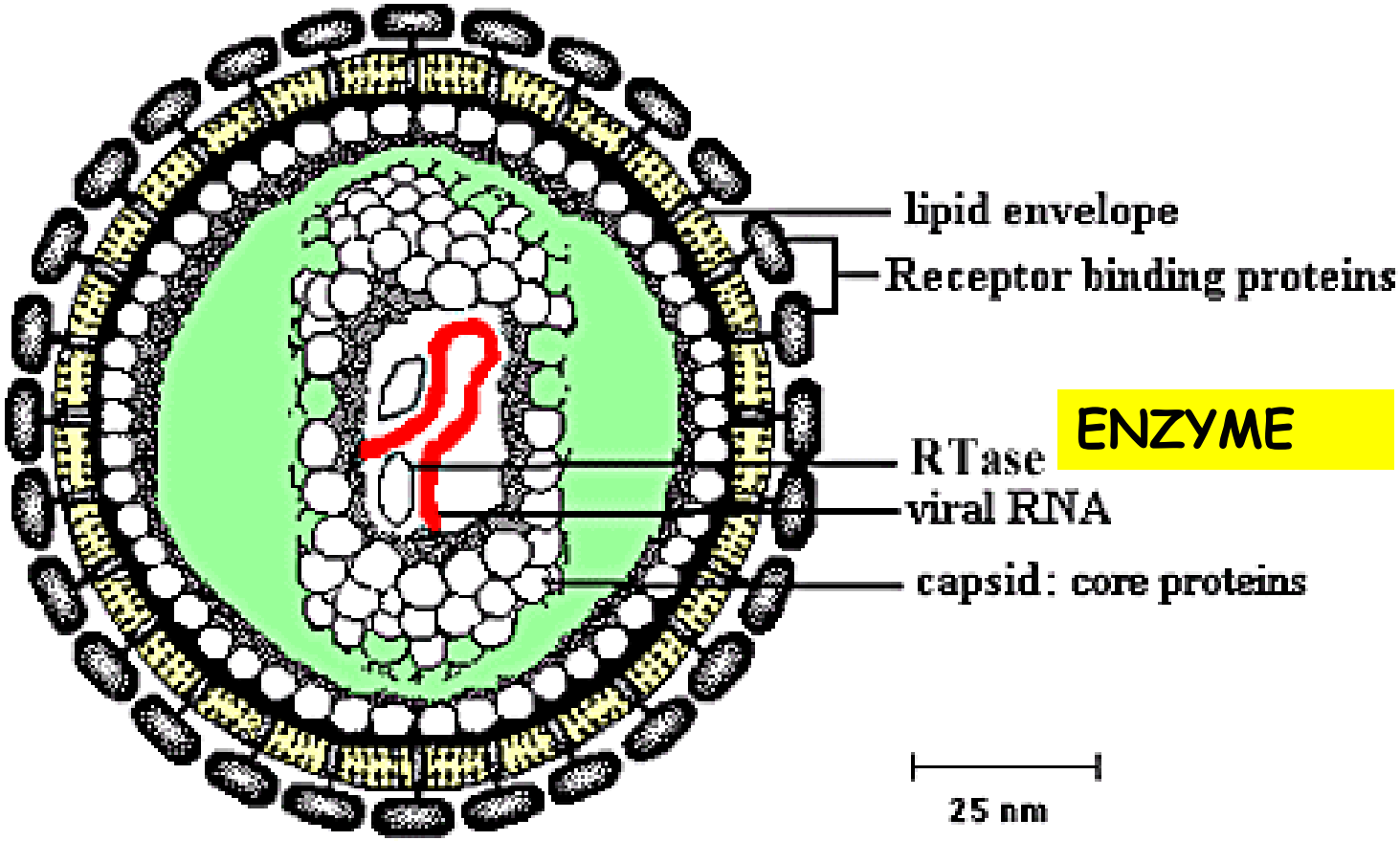
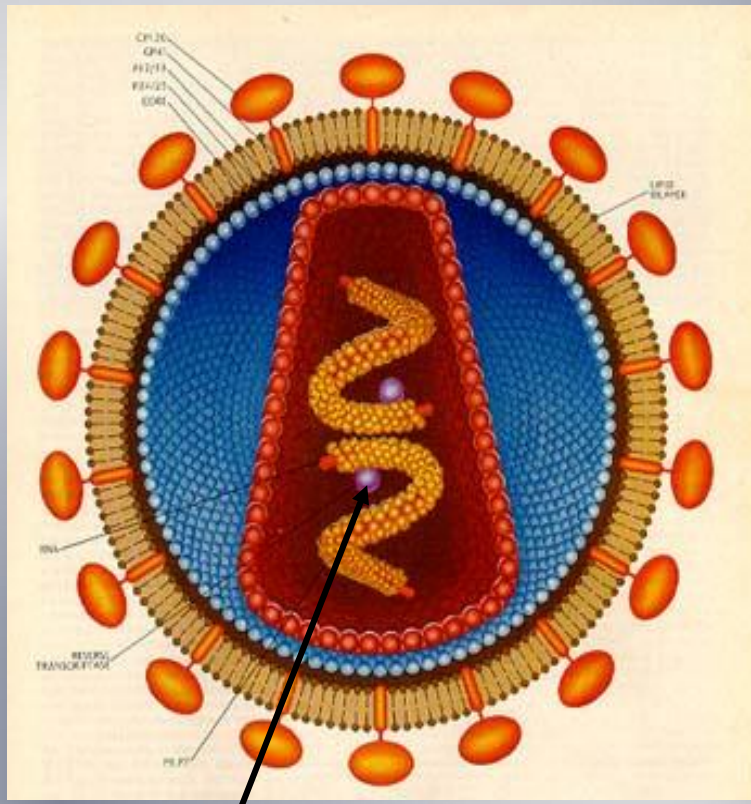


Diagram of a Retrovirus

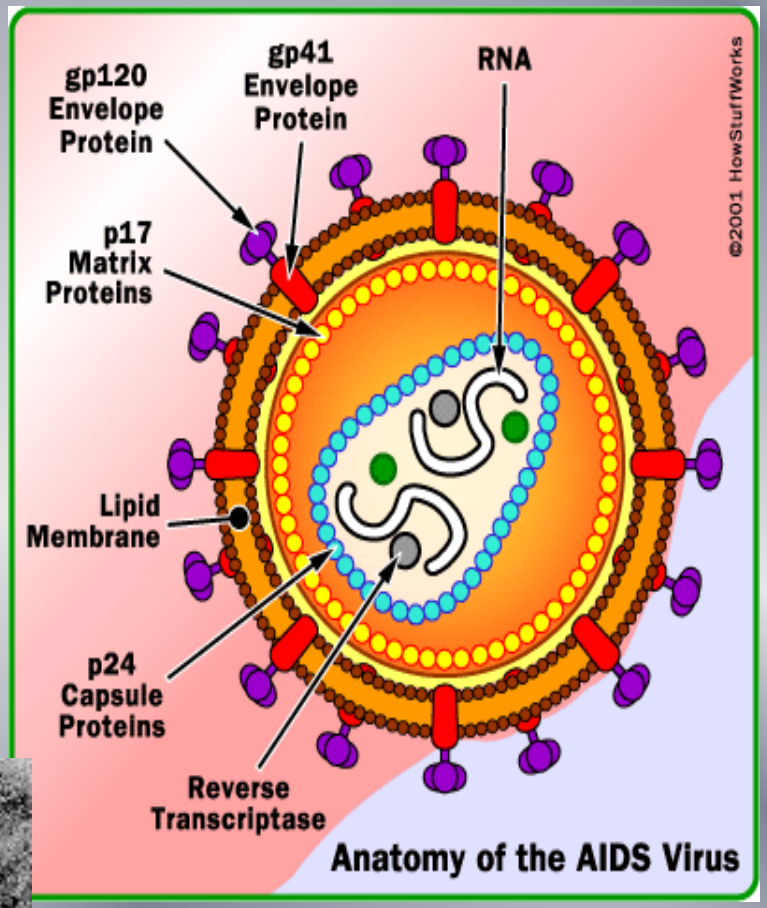
Retroviruses

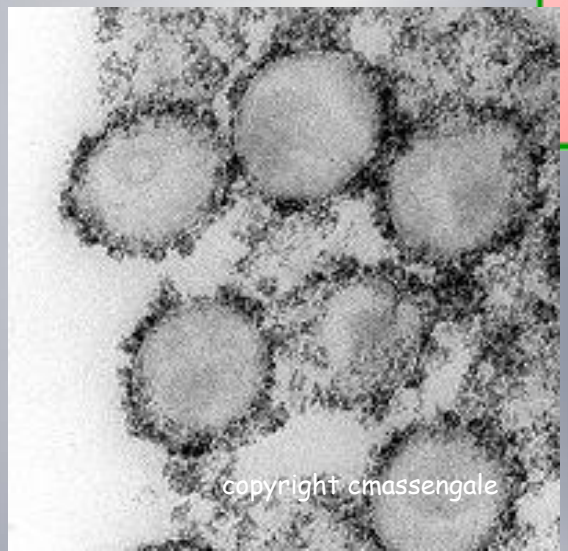
- The enzyme reverse transcriptase (or RTase), which causes synthesis of a complementary DNA molecule (cDNA) using virus RNA as a template



Retroviruses

- HIV, the AIDS virus, is a retrovirus
- Feline Leukemia Virus is also a retrovirus



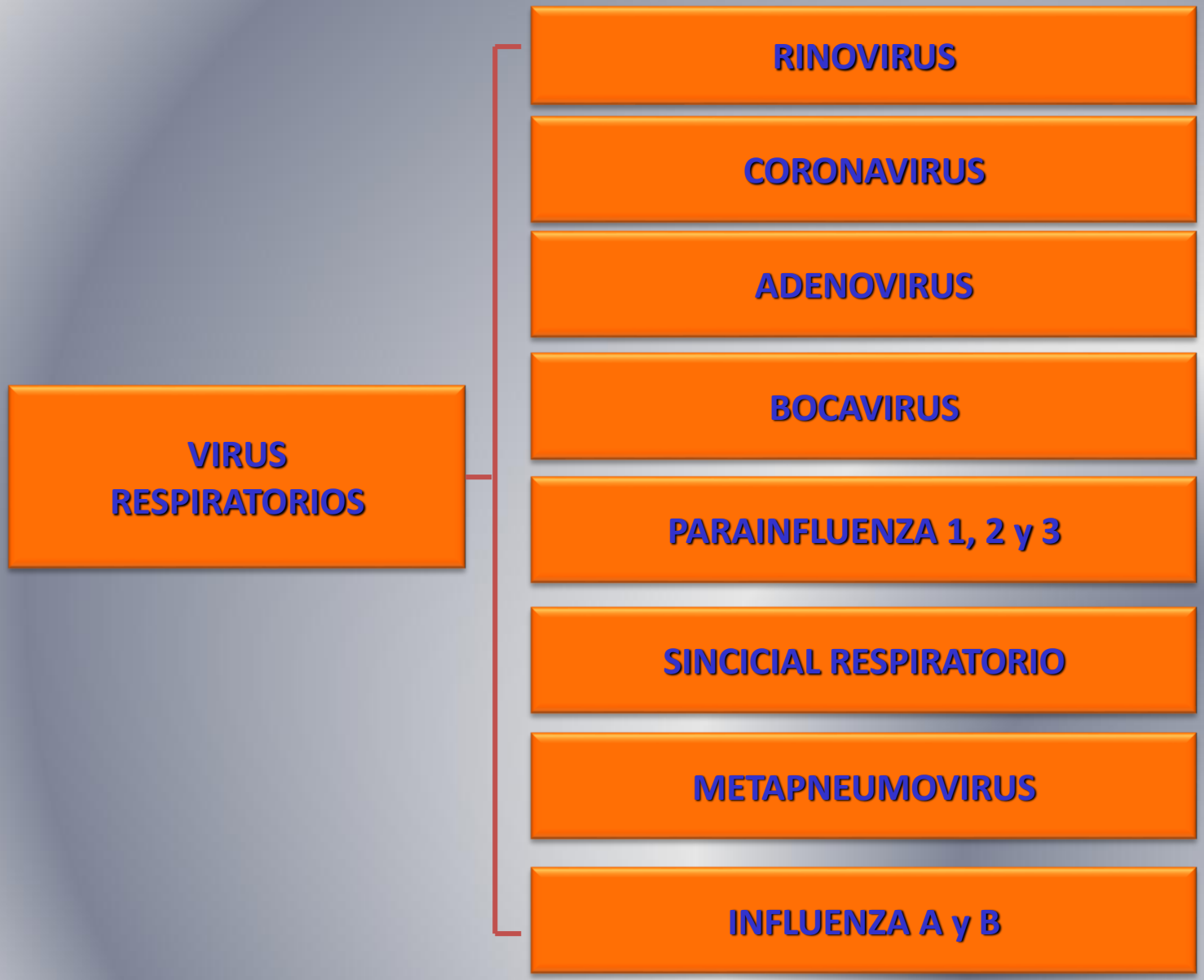


Viroids

- Small, circular RNA molecules without a protein coat
- Infect plants
- Potato famine in Ireland

"VIRUS RESPIRATORIOS: CUÁNTOS HAY

- **Rinovirus: A, B y C; > 100 serotipos**
- **Coronavirus: *Alfa*: 229E, NL63 *Beta*: OC 43, HKU1, SARS; MERS**
- **VRS: grupos A y B; lineajes, genotipos.**
- **Metapneumovirus: A y B; subgrupos A1, A2, B1, B2**
- **Adenovirus: 55 serotipos; muchos genotipos**
- **Influenzas : subtipos A, B, C; cepas.**
- **Parainfluenza: 1- 4.**
- **Otros: bocavirus, enterovirus, CMV, sarampión, varicela, hantavirus.**


VIRUS RESPIRATORIOS
RINOVIRUS
CORONAVIRUS
ADENOVIRUS
BOCAVIRUS
PARAINFLUENZA 1, 2 y 3
SINCICIAL RESPIRATORIO
METAPNEUMOVIRUS
INFLUENZA A y B

Infecciones respiratorias y agentes etiológicos virales.

Sindrome	Virus Más frecuentes	Menos frecuentes
Infección respiratoria alta	Rinovirus Coronavirus Adenovirus Parainfluenza 3	Influenza A o B Parainfluenza 1 o 2 V.R.S Enterovirus
Faringitis	Adenovirus V.Epstein-Barr Enterovirus V.Herpes Simplex	Influenza A o B V.R.S. Parainfluenza 1 y 2 Rinovirus Coronavirus
Croup	Parainfluenza 1,2,3	Influenza A V.R.S. Sarampión Coronavirus
Bronquiolitis	V.R.S.	Adenovirus Parainfluenza 1 y2 Influenza A o B Rinovirus
Neumonia	V.R.S. Parainfluenza 3 Adenovirus Influenza A	Parainfluenza 1 y 2 Rinovirus V.Epstein-Barr

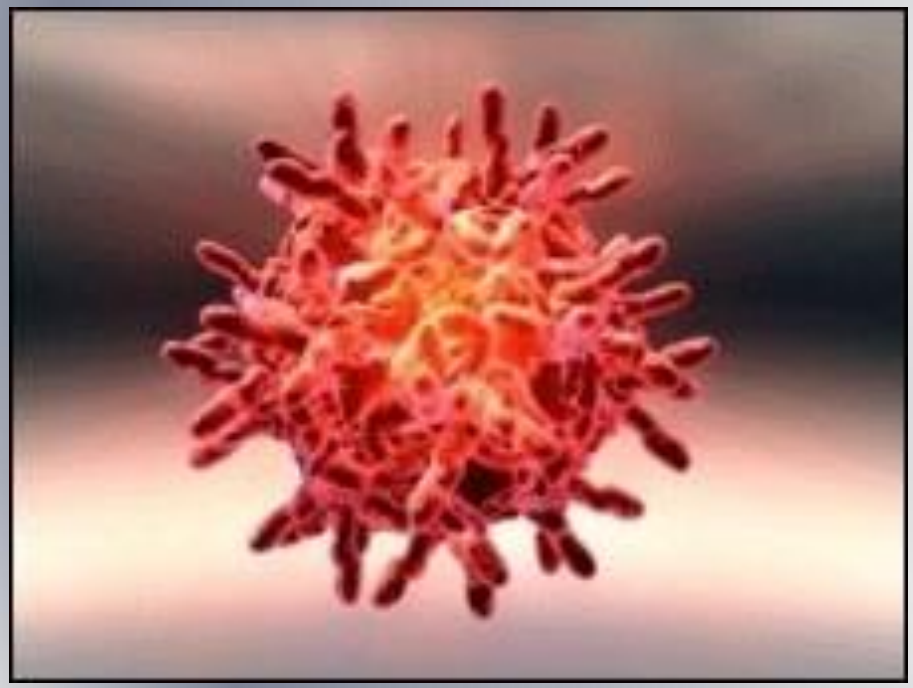
- **Resfrío común** representa un tercio o la mitad de infecciones respiratorias agudas en humanos.

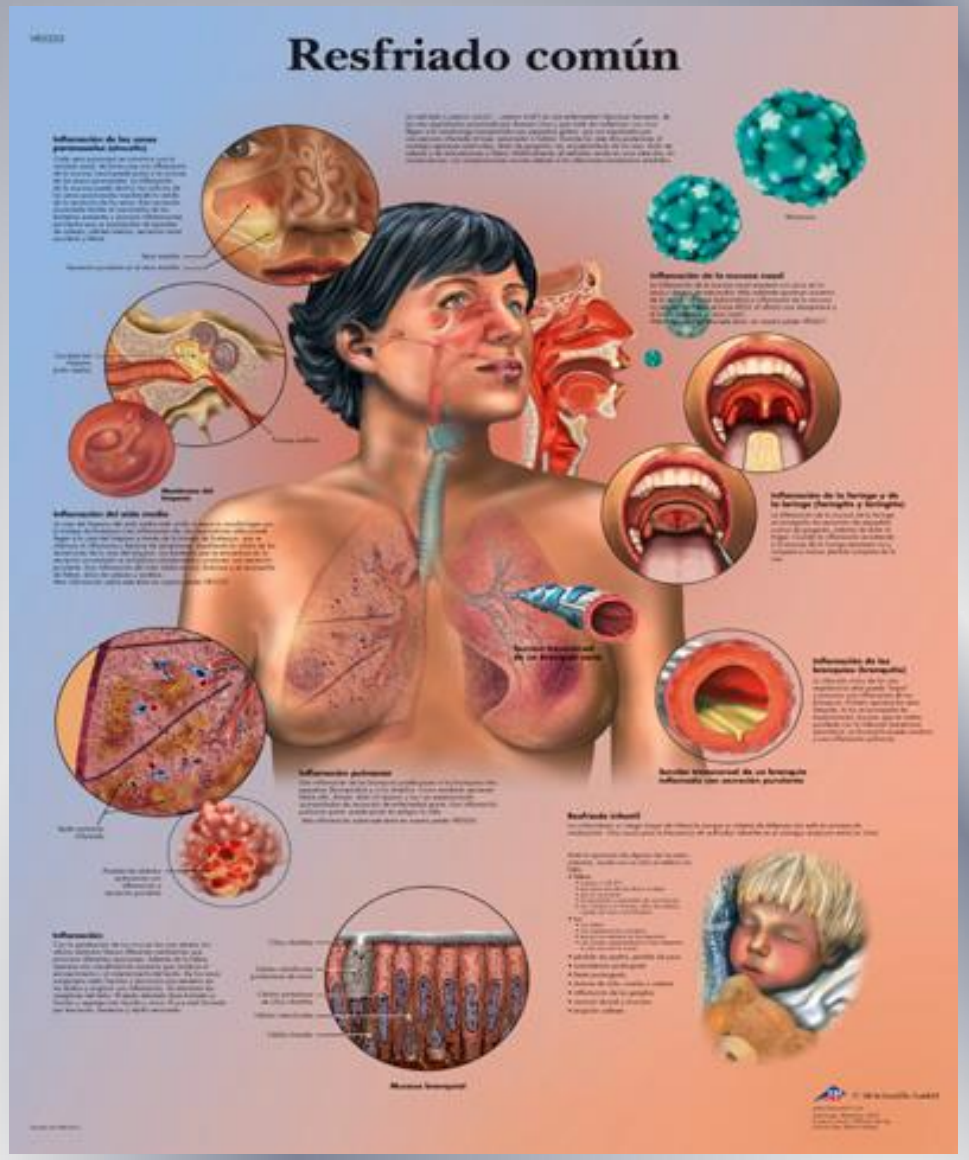
- **Rinovirus** responsables del 30-50% de los resfríos comunes, y **coronavirus** 10-30%.

- El resto es debido a adenovirus, enterovirus, RSV, influenza, y parainfluenza, los que pueden dar síntomas indistinguibles a los que dan rino y coronavirus.

RINOVIRUS

- Virus RNA ss
- Familia: picornaviridae
- ácido-lábil
- **> de 100 serotipos.**

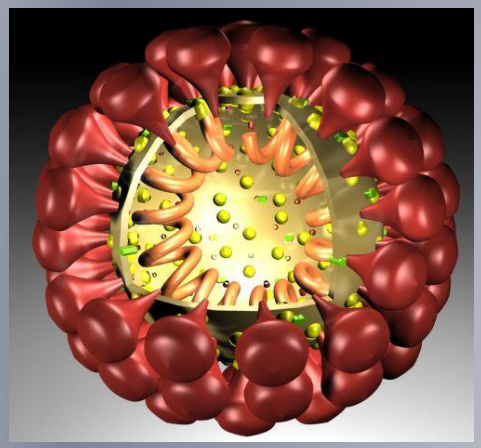


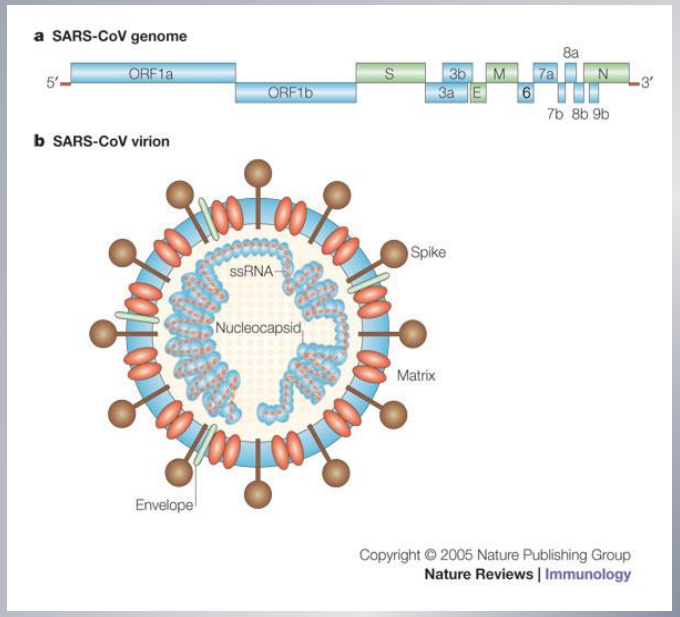


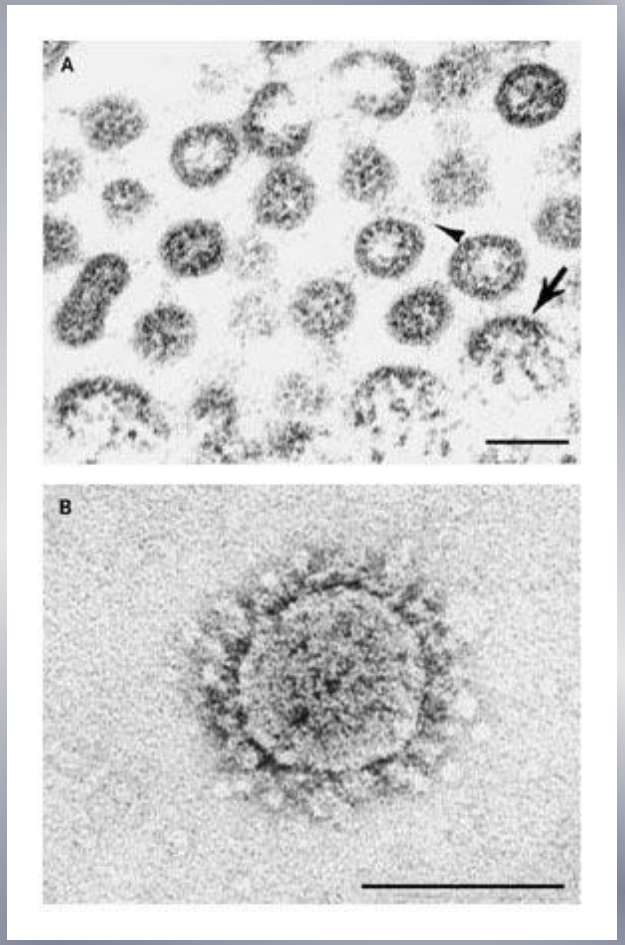
CORONAVIRUS

Esféricos, Envueltos de 80 a 220 nm

Multiplicación: citoplasma, salida por gemación.







Proteínas: Matriz protéica (M), glicoproteína (S) – aspecto de corona solar, nucleocapside (N), hemaglutinina-esterasa (HE).

Heterogeneidad antigénica significativa.

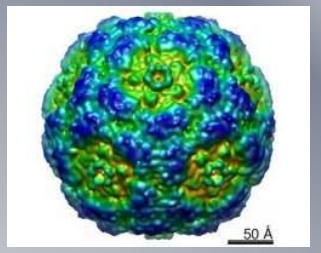
Reacciones cruzadas antigénica entre cepas humanas y animales.

BOCAVIRUS HUMANO HBoV

Los virus de la familia Parvoviridae son de pequeño tamaño (20-26 nm), de simetría icosaédrica y no poseen manto, su genoma está **formado por ADN de simple hebra** y es dependiente de células en división para la replicación.

Subfamily	Genus	Species
Parvovirinae	*Dependovirus*	Adeno-associated virus 2
	Parvovirus	Minute virus of mice Feline panleukopenia virus
	Erythrovirus	B 19 virus
	Bocavirus	Human bocavirus
Densovirinae	*Iteravirus*	*Bombyx mori* densovirus

BOCAVIRUS HUMANO HBoV



Descrito por primera vez en el año 2005 identificado por métodos moleculares en muestras de aspirado nasofaríngeo en niños con infecciones del tracto respiratorio inferior.

Las secuencias genéticas y el análisis filogenético **muestran una estrecha relación de HBoV con dos miembros de la familia Parvoviridae: parvovirus bovino (BPV) y virus minute canino (CMnV),** por lo que recibió el nombre provisorio de bocavirus humano (HBoV), "bo" de bovino y "ca" de canino.

Se describe una **prevalencia de 2,7% a 19% (Suecia) principalmente en niños menores a dos años de edad.** Estas diferencias pueden explicarse por la estacionalidad, predominando en invierno, y por las distintas poblaciones de los estudios.

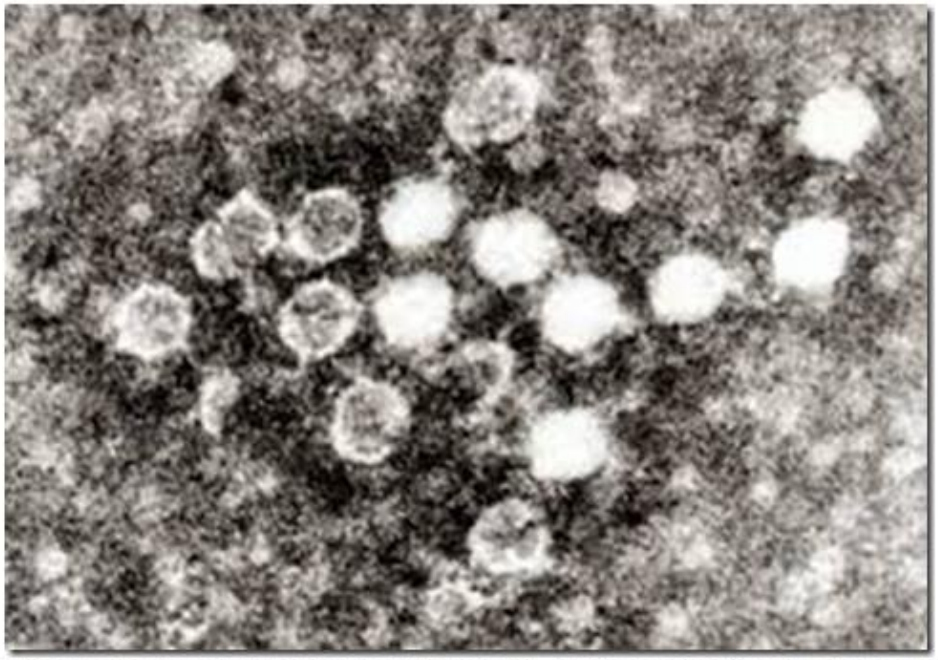
HBoV y B19V serían los dos únicos miembros de esta familia viral que causan enfermedades en humanos: **B19V responsable de eritema infeccioso en niños e hidrops fetal en infecciones intrauterinas y HBoV como agente viral de infecciones del tracto respiratorio, principalmente en niños bajo 5 años de edad.**

BOCAVIRUS HUMANO HBoV

Supuesto Bocavirus humano provoca dos primeras muertes en Bolivia

31/10/2013 publicado por Omar Pereyra 0 Archivado en:Vida

El Bocavirus humano (HBoV) habría provocado la muerte de dos niños menores de un año, las primeras a causa del mal en Bolivia, informó este jueves una fuente médica del Ministerio de Salud.



"Se ha confirmado que el agente asociado a estas muertes es el Bocavirus. Son dos pacientes que están entre 7 y 8 meses de edad", dijo el médico Rubén Colque, director del servicio de Salud de esa repartición.

"Es la primera vez que se identifica este agente causal de muerte" en Bolivia, remarcó.

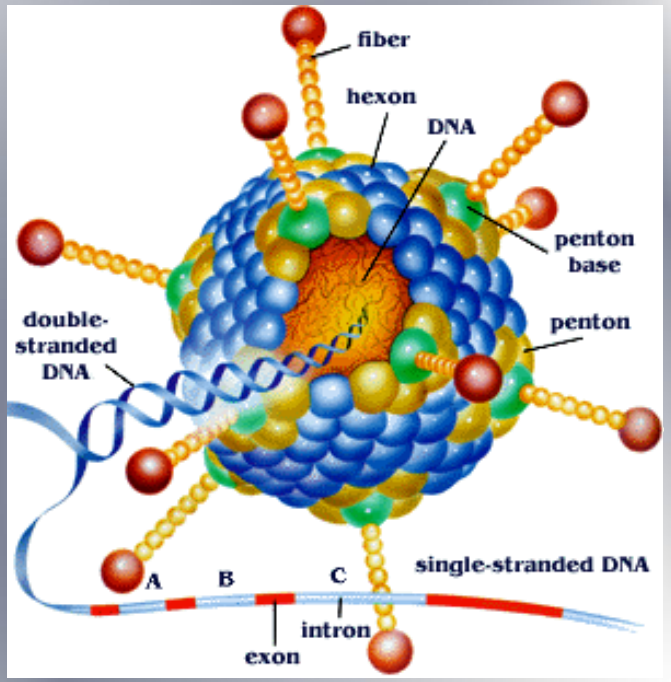
ADENOVIRUS

Adenoviridae: ADN doble cadena, lineal, asociado a polipéptidos V y VII formando un core.

Simetría icosaédrica. Sin envoltura

Partícula es estable a pH bajos y resiste secreciones gástricas y biliares.

Existen 6 subgéneros con distintos tipos.



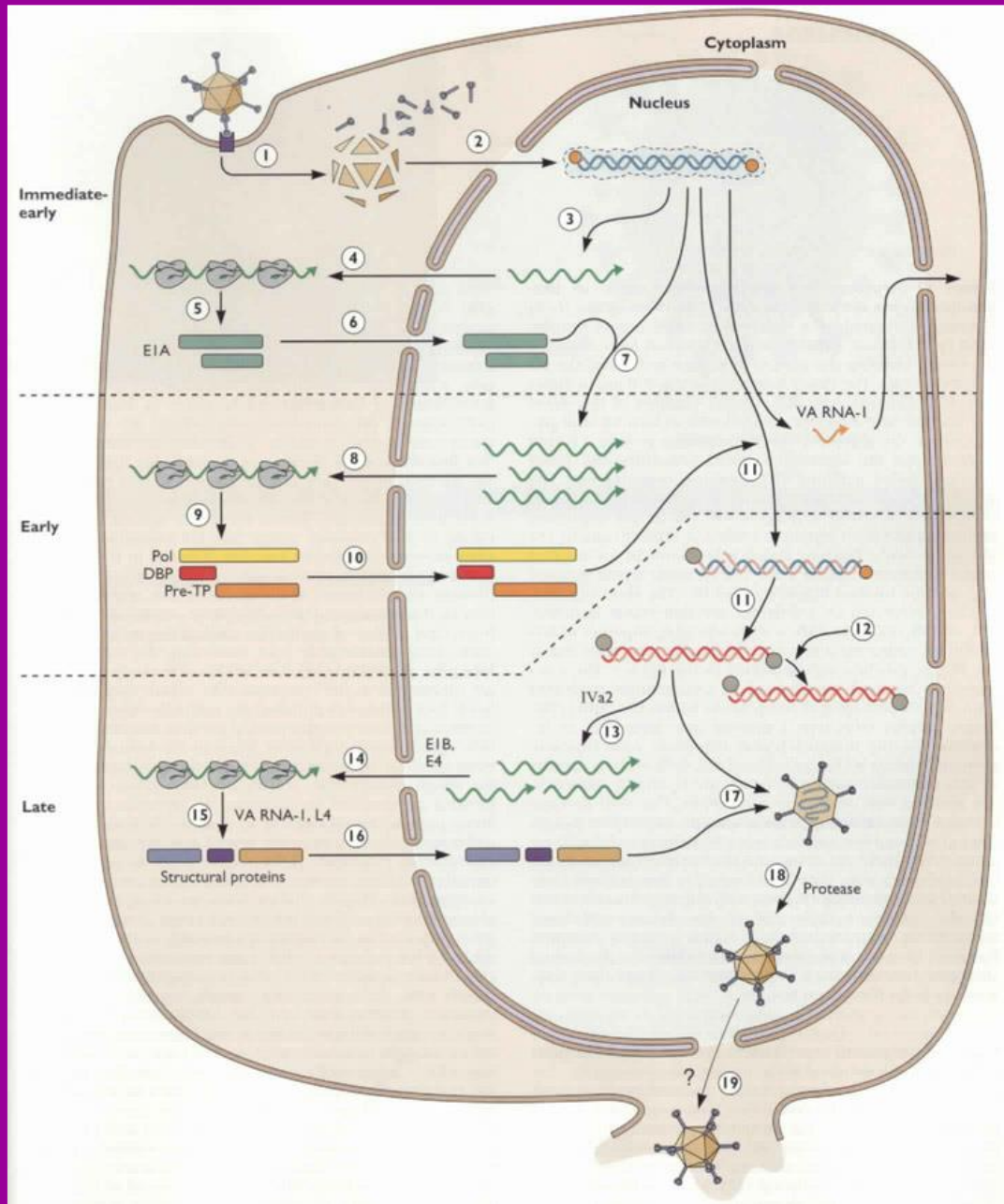
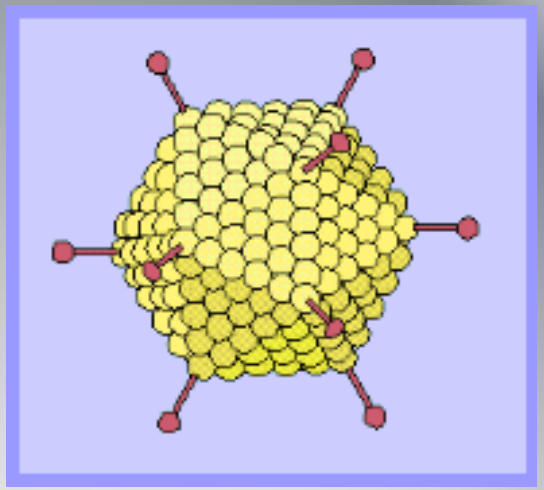


Figure 16 Single-cell reproductive cycle of human adenovirus type 2.

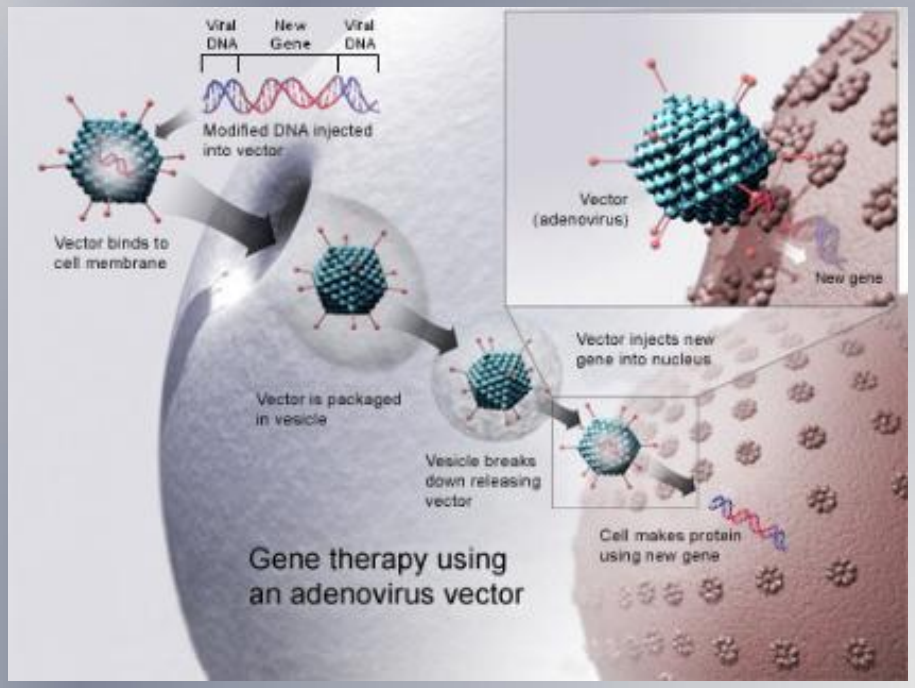
ADENOVIRUS

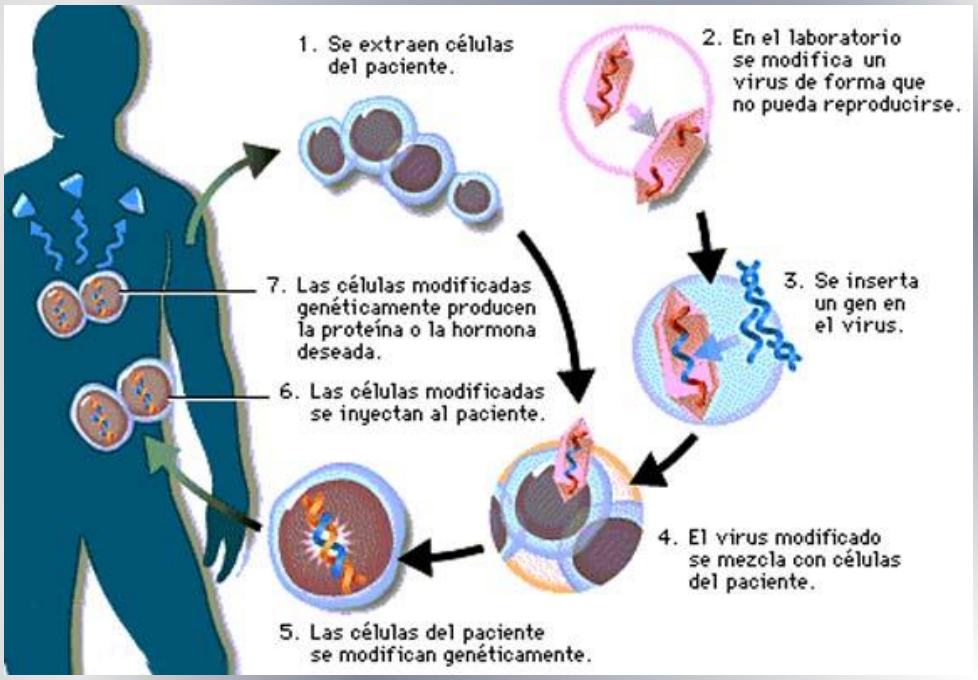


Transmisión por contacto directo
Incubación: 2 – 14 días
Sindromes de infección respiratoria alta y baja
Aislamiento a partir de secreciones respiratorias
Fiebre faringoconjuntival
Infecciones oculares: 3, 7 y 4.
Infecciones intestinales: tipos 3 y 7.

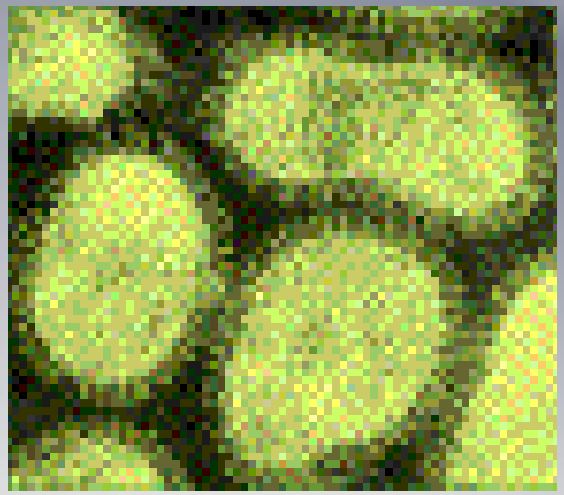
Endémico en toda la población durante todo el año
Causa epidemias invernales
Altamente recidivante

Terapia génica con ADV





PARAINFLUENZA



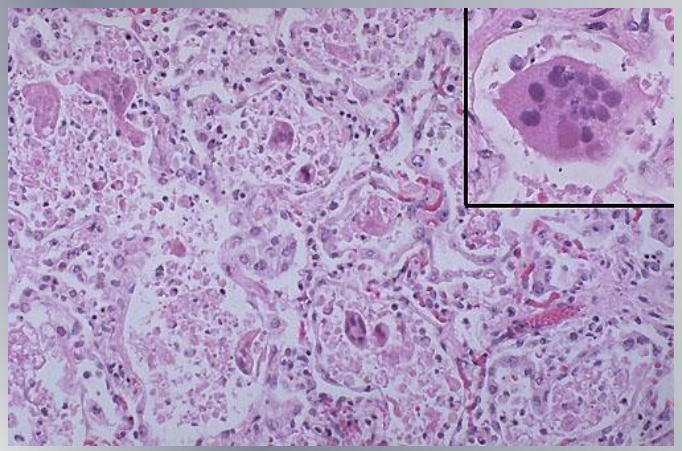
RNA virus, Paramyxoviridae, Género: Paramixovirus
Esféricos, pleomórficos por envoltura laxa.
Nucleocápside helicoidal con **ARN polaridad (–)**
Genoma no segmentado, Transmisión por contacto directo.
Principal causa de **crup (laringo-traqueo-bronquitis) en niños, neumonia y bronquiolitis en menores 6 meses**
Circulan todo el año

PROTEINAS:

-**Glucoproteínas**: ubicadas en espículas que contiene actividad de **Neuraminidasa y Hemaglutinina**.
-**Proteína de fusión**: permite penetración por fusión con la membrana celular
-**Proteína M**: en parte interna virión.

SINCICIAL RESPIRATORIO

RNA virus, Paramyxoviridae. Género: Pneumovirus
Solo afecta al hombre.
Provoca formación de sincicios en células.
Carece de Hemaglutinina.



SINCICIAL RESPIRATORIO

Transmisión por contacto directo. Reinfecciones durante toda la vida

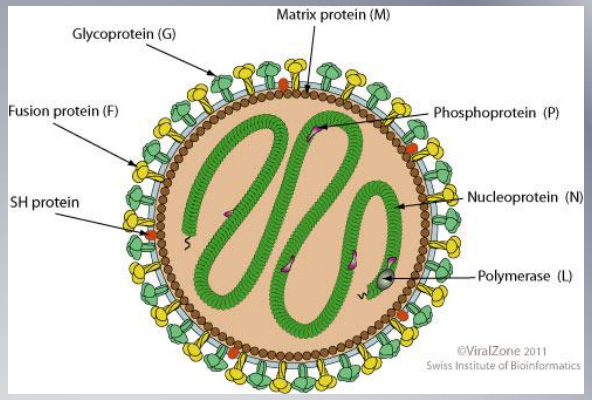
Principal causa de neumonía y bronquiolitis en niños menores de 2 años

Patógeno importante en inmunocomprometidos

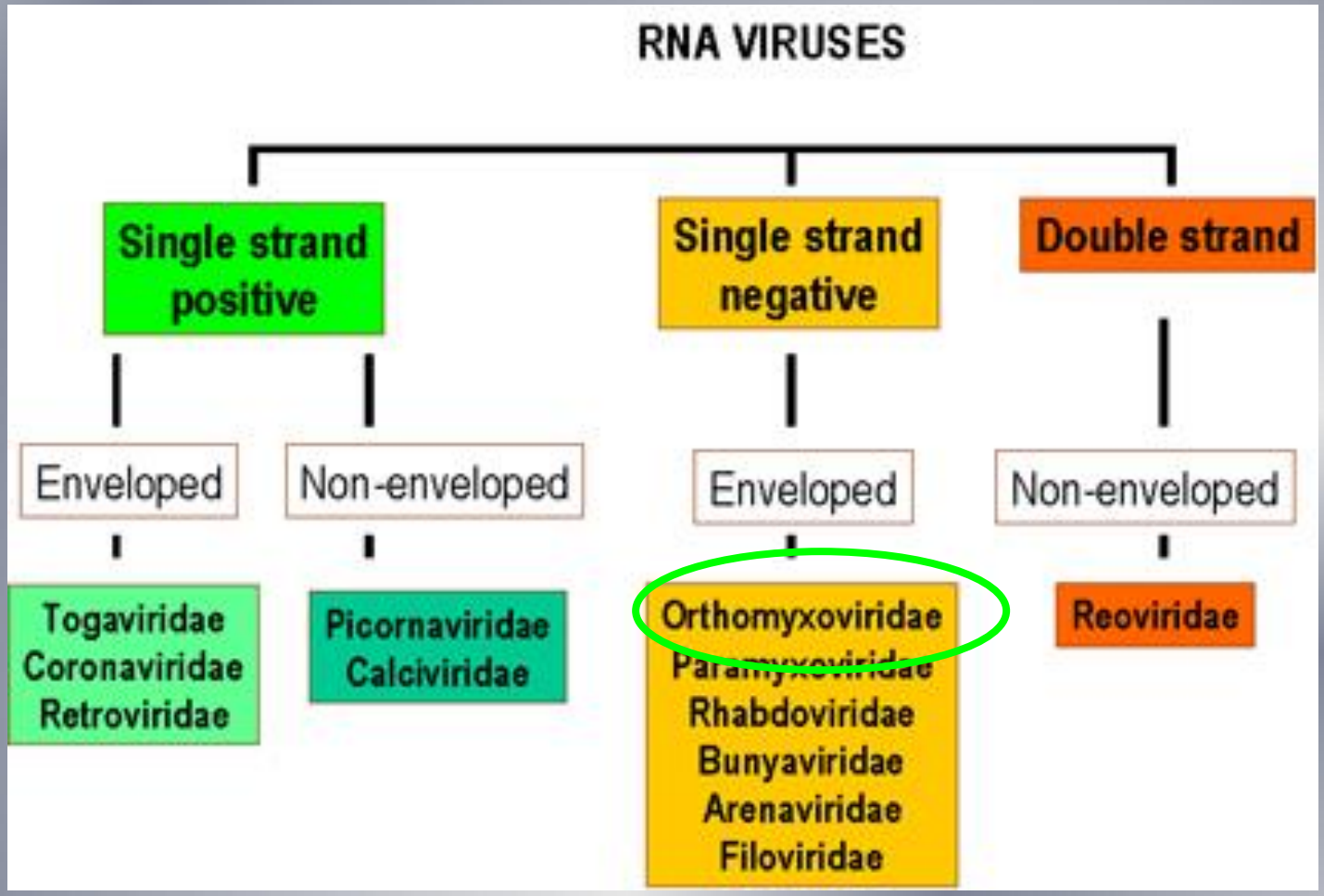
Causa de epidemia de neumonías en ancianos hospitalizados.

Causa brotes estacionales todos los años.

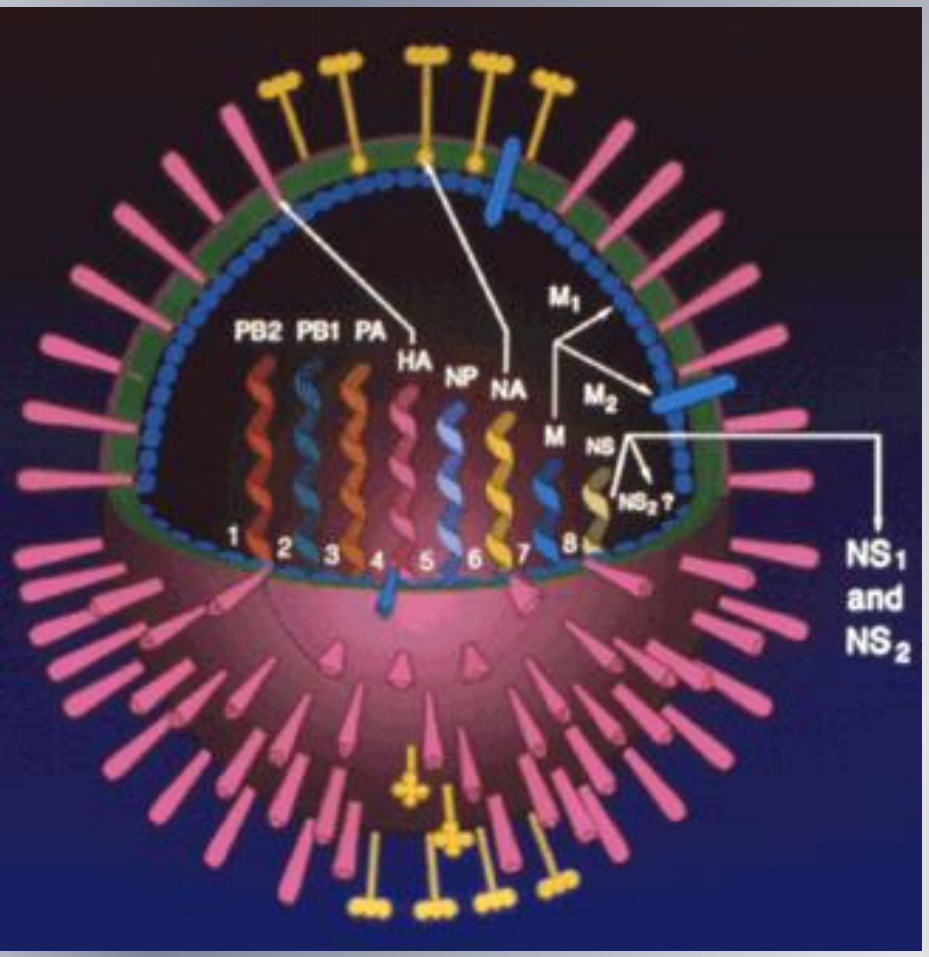
METAPNEUMOVIRUS hMPV



- Subfamilia: *Pneumovirinae*.
- Género: *Metapneumovirus.*
- Aislado por primera vez en 2001 en Holanda.
- Responsable de un 5% de las infecciones respiratorias en niños menores de 5 años.
- Es uno de los 1ros o 2dos microorganismos más frecuentemente aislados.
- Se presenta en coinfección con RSV.
- Infecciones respiratorias altas
- Otitis media
- Laringotraqueobronquitis
- Bronquiolitis
- Neumonia
- Posibilidad de neumonias fatales en transplantados.

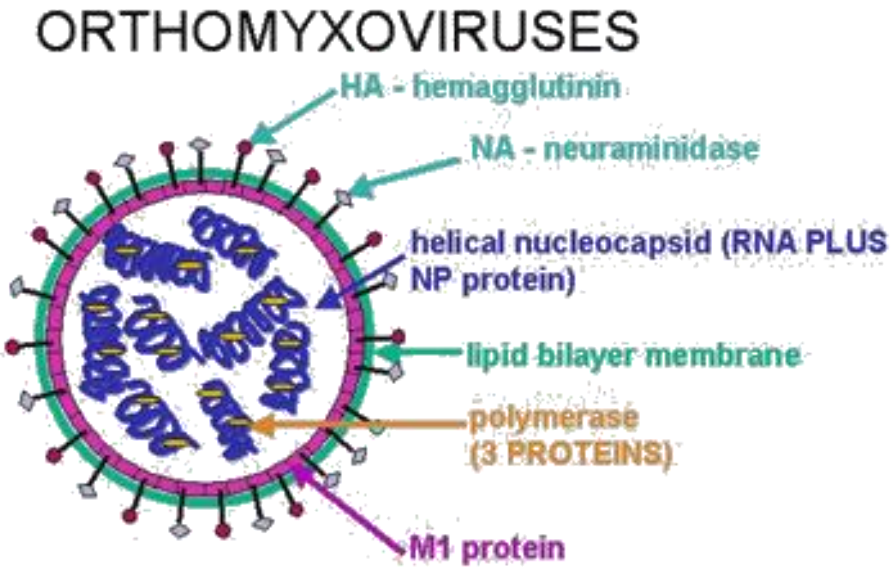

RNA VIRUSES
Single strand positive
Single strand negative
Double strand
Enveloped
Non-enveloped
Enveloped
Non-enveloped
Togaviridae
Coronaviridae
Retroviridae
Picornaviridae
Calciviridae
Orthomyxoviridae
Rhabdoviridae
Bunyaviridae
Arenaviridae
Filoviridae
Reoviridae

ORTHOMYXOVIRIDAE

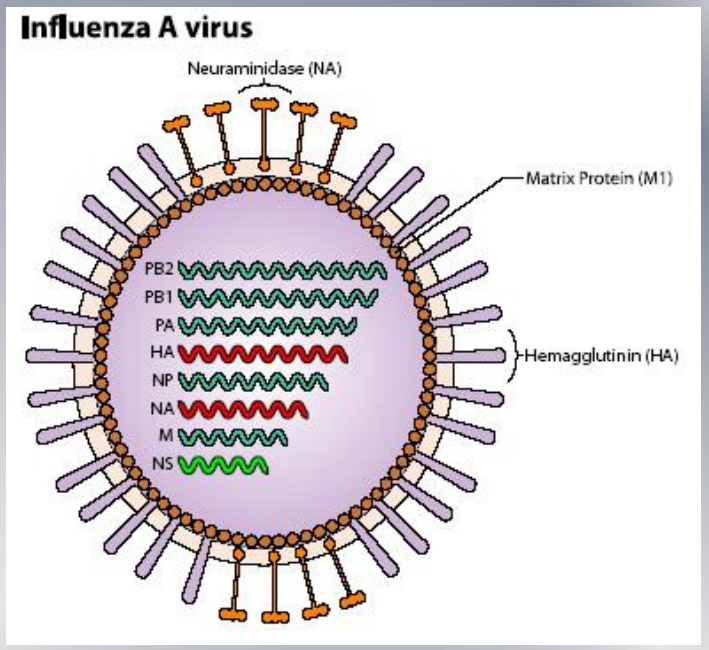


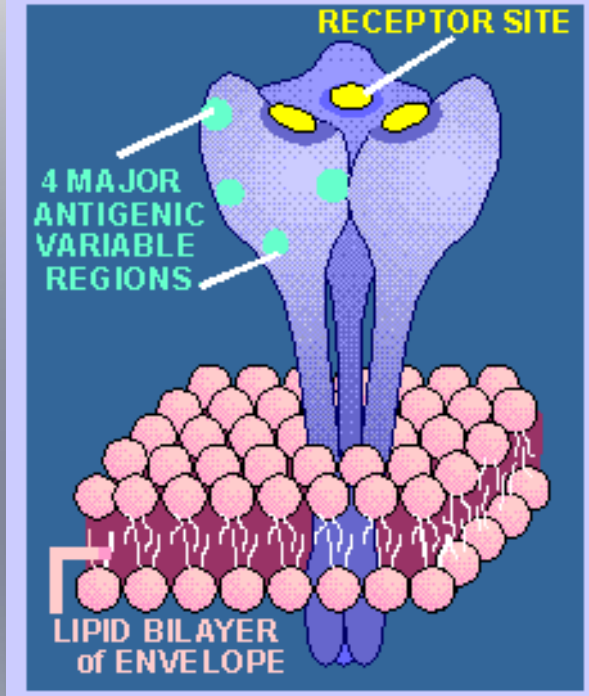
- Virus Envuelto , 80-120 nm diametro
- Particulas morfologicamente variables
- ARN simple cadena sentido negativo
- Genoma segmentado
  - (8 seg en IA e IB)
  - (7 seg en IC)
- Glicoproteínas de Superficie:
  - Hemaglutinina
  - Neuraminidasa
- Proteinas Estructurales:
  - Nucleoproteinas (NP)
  - Matrix (M)

INFLUENZA



type A, B, C : NP, M protein
sub-types: HA or NA protein





RNA virus, Orthomyxoviridae.
Genoma segmentado en 8
cadena lineal y polaridad positiva

FAMILIA: *Orthomyxoviridae*

Genero	Especie/tipo	Hospedero
Influenza virus A	***Influenza A virus*** **HxNx**	**Vertebrados**
Influenza virus B	***Influenza B virus***	**Vertebrados**
Influenza virus C	***Influenza C virus***	**Vertebrados**
Thogotovirus	*Thogoto virus* *Dhori virus*	Vertebrados
Isavirus	*Infectious salmon anemia virus*	Vertebrados

INFLUENZA

Tipo A:

Afecta hombre y equinos, porcinos, focas, aves domésticas y silvestres.

Enfermedad moderada a severa

Produce pandemias y epidemias

Existen dos subtipos circulantes H3N2 y H1N1

Tipo B:

Enfermedad más leve o similar

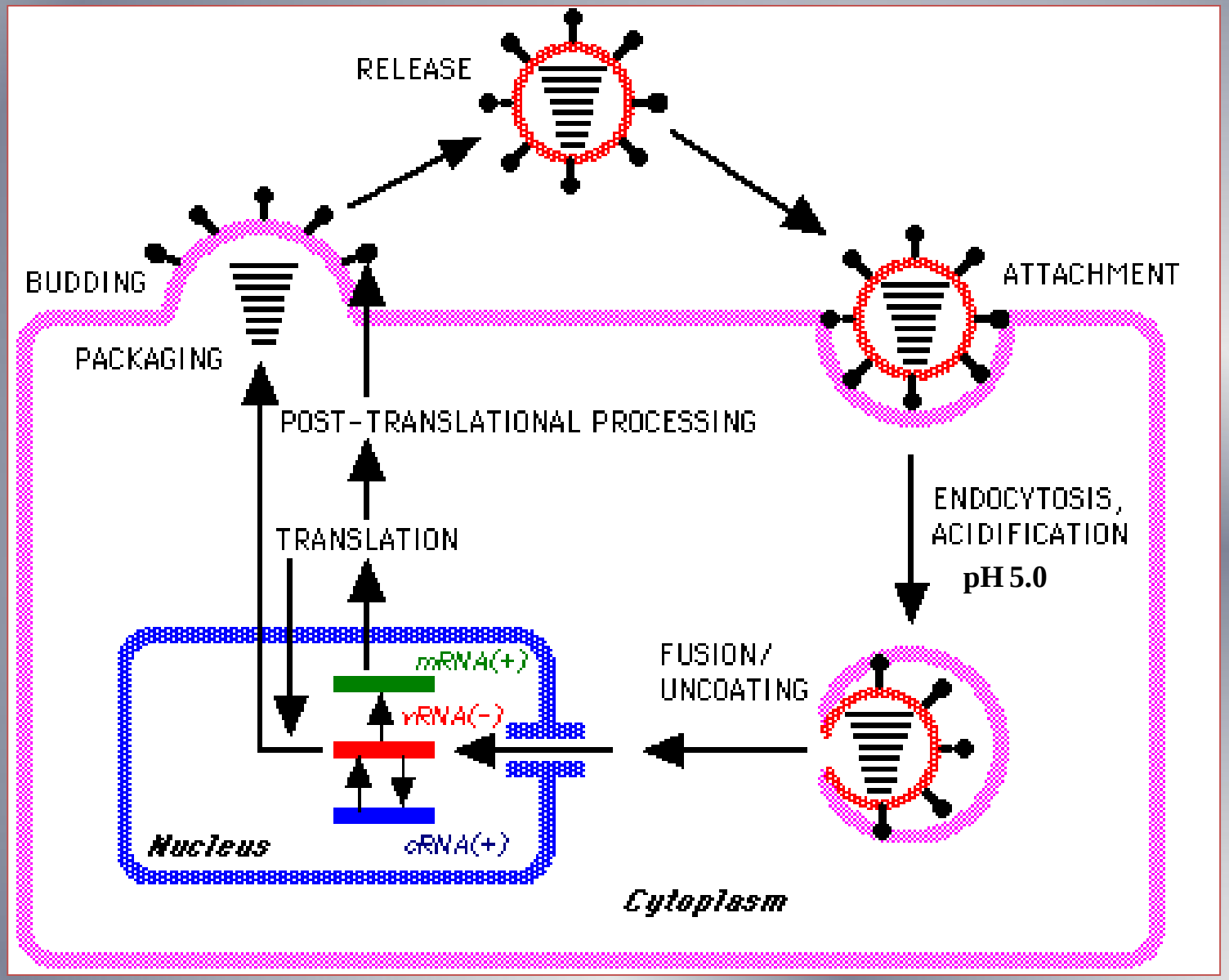
No tiene subtipos

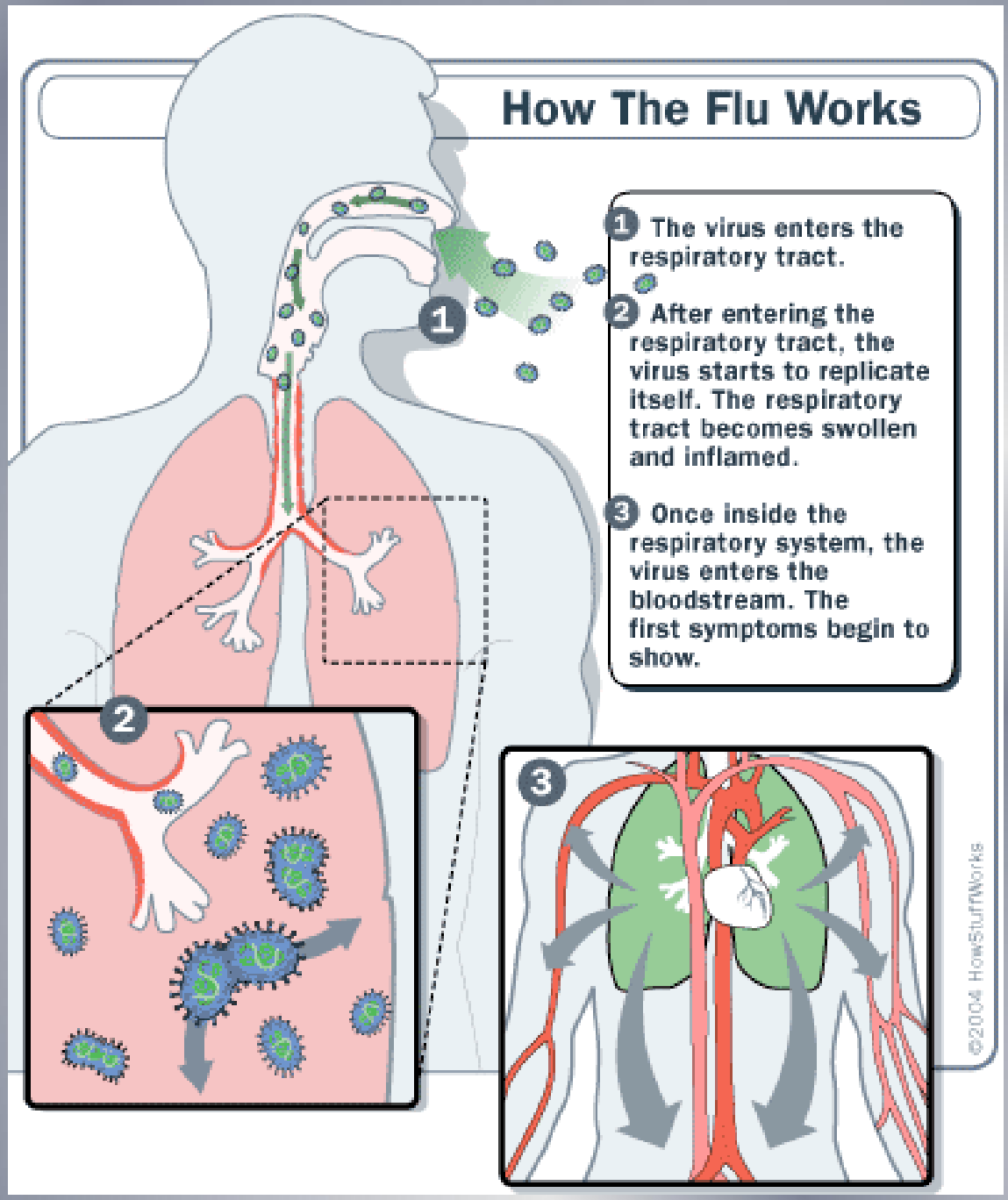
Asociado a Sind de Reyé en niños

Tipo C:

Mayoría de casos subclínicos

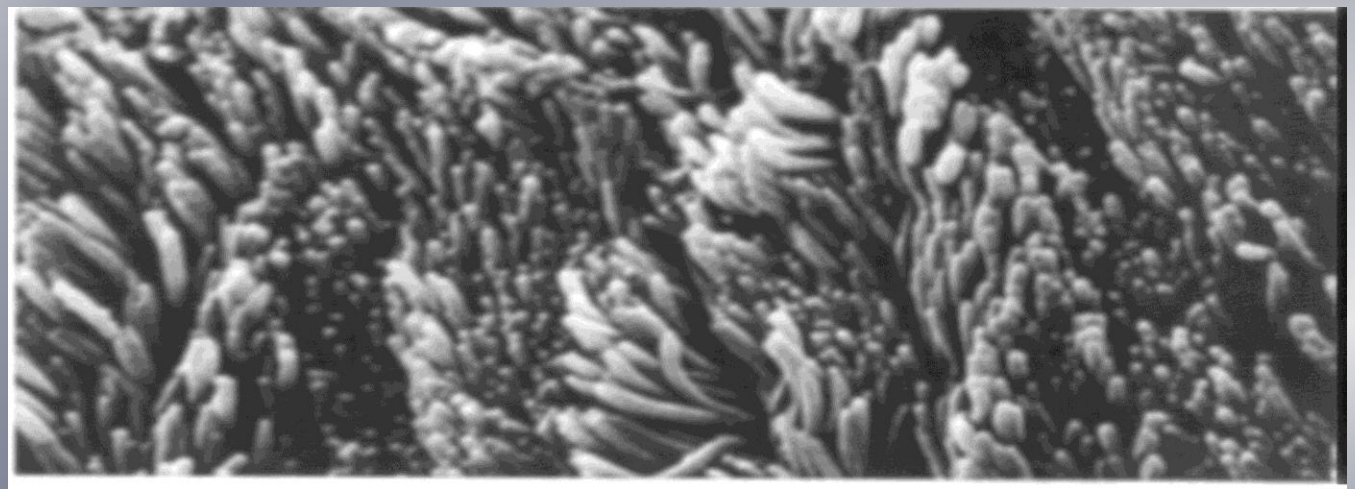
CICLO REPLICATIVO DE INFLUENZA



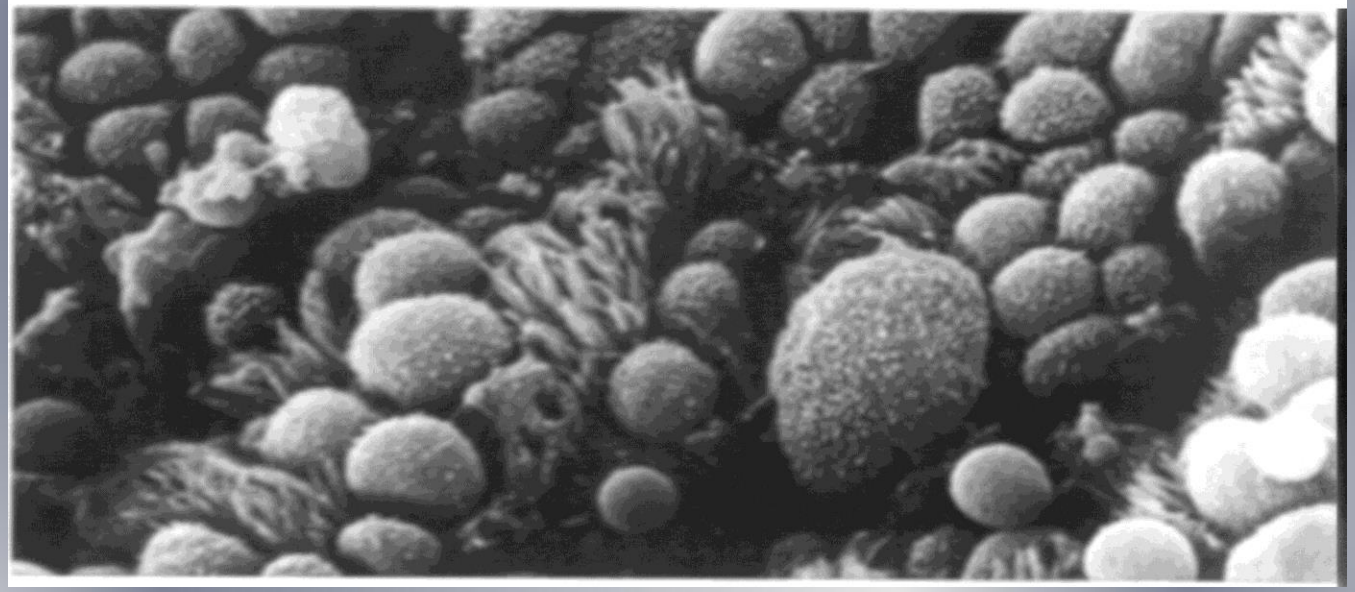

How The Flu Works
1 The virus enters the respiratory tract.
2 After entering the respiratory tract, the virus starts to replicate itself. The respiratory tract becomes swollen and inflamed.
3 Once inside the respiratory system, the virus enters the bloodstream. The first symptoms begin to show.
1
2
3
©2004 HowStuffWorks

INFLUENZA

Patogenia



Mucosa epitelial sana



Mucosa epitelial infectada con Influenza A

INFLUENZA

COMPLICACIONES:

- **RESPIRATORIAS**: neumonía viral primaria, neumonía bacteriana secundaria y neumonía mixta.

- **NEUROLOGICAS**: encefalitis, polineuritis, Guillen Barré, sindrome de Reyé, convulsiones.

- **CARDIACAS**: alteraciones funcionales.

VARIACION ANTIGENICA

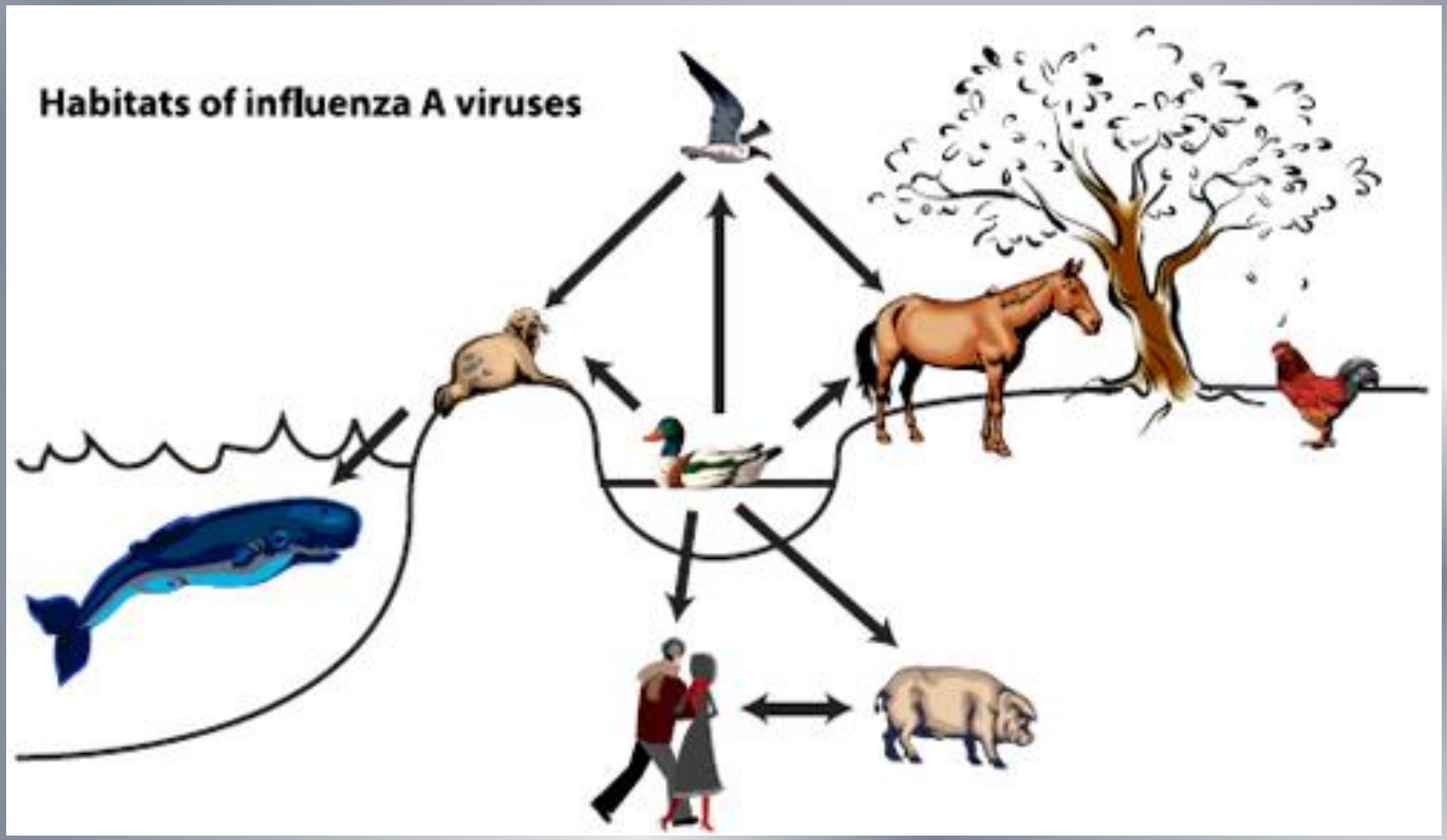
MAYORES: **SHIFT**

Solo descriptas para tipo A. Cambios en genes que codifican HA y NA. El virus aparece con HA y/o NA diferente al que circuló antes. Causan pandemias.

MENORES: **DRIFT (Deriva)**

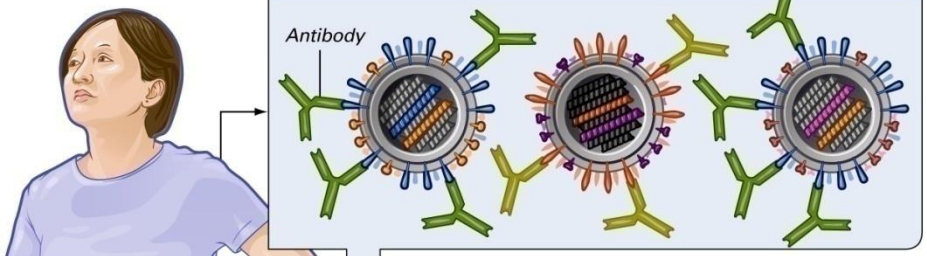
Afecta a tipo A y B. Se producen de manera gradual cada 2 a 3 años dentro de un mismo tipo o de un subtipo. Mutaciones en el ARN viral que se traducen en cambios en AA en los polipéptidos.

INFLUENZA



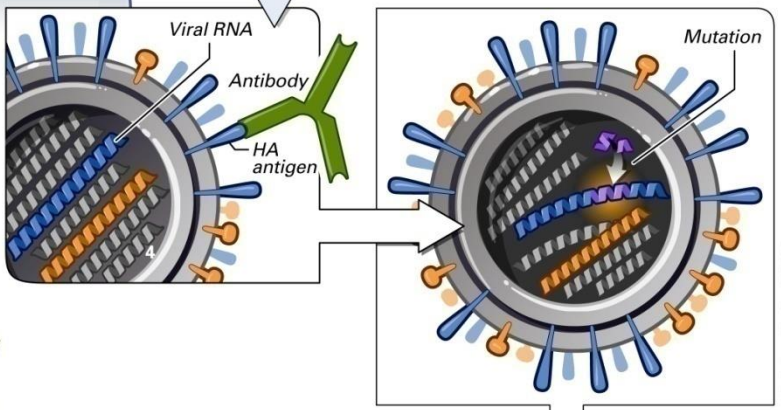
1 Each year's flu vaccine contains three flu strains – two A strains and one B strain – that can change from year to year.

2 After vaccination, your body produces infection-fighting antibodies against the three flu strains in the vaccine.



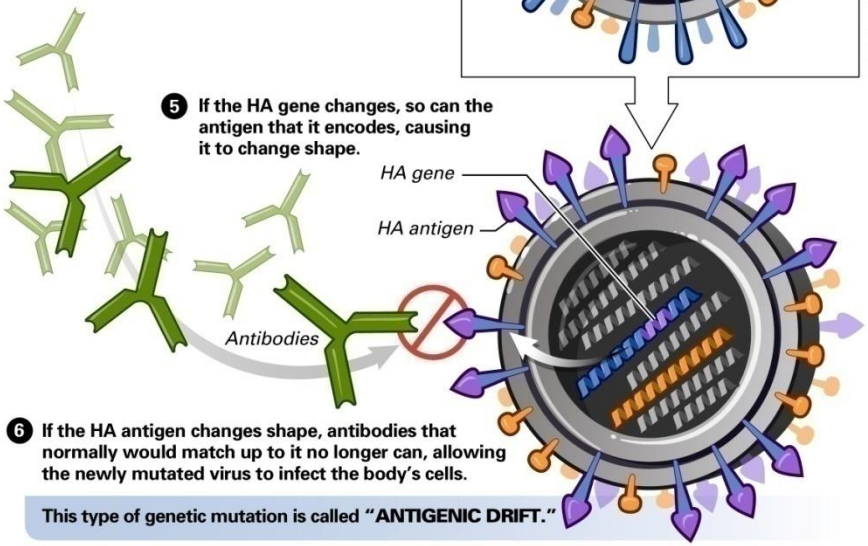
3 If you are exposed to any of the three flu strains during the flu season, the antibodies will latch onto the virus's HA antigens, preventing the flu virus from attaching to healthy cells and infecting them.

4 Influenza virus genes, made of RNA, are more prone to mutations than genes made of DNA.



Link Studio for NIAID

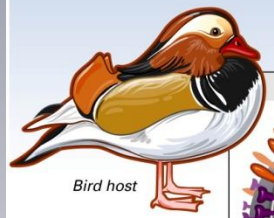
5 If the HA gene changes, so can the antigen that it encodes, causing it to change shape.



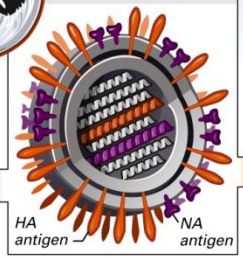
6 If the HA antigen changes shape, antibodies that normally would match up to it no longer can, allowing the newly mutated virus to infect the body's cells.

This type of genetic mutation is called **"ANTIGENIC DRIFT."**

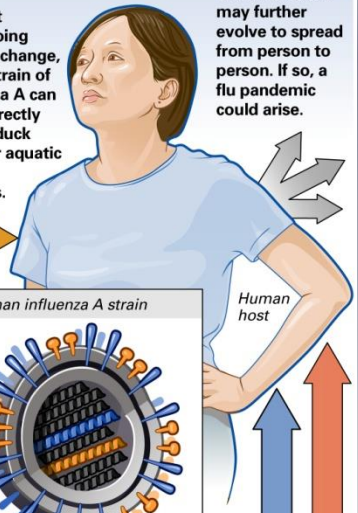
The genetic change that enables a flu strain to jump from one animal species to another, including humans, is called **"ANTIGENIC SHIFT."** Antigenic shift can happen in three ways:



B Without undergoing genetic change, a bird strain of influenza A can jump directly from a duck or other aquatic bird to humans.



The new strain may further evolve to spread from person to person. If so, a flu pandemic could arise.

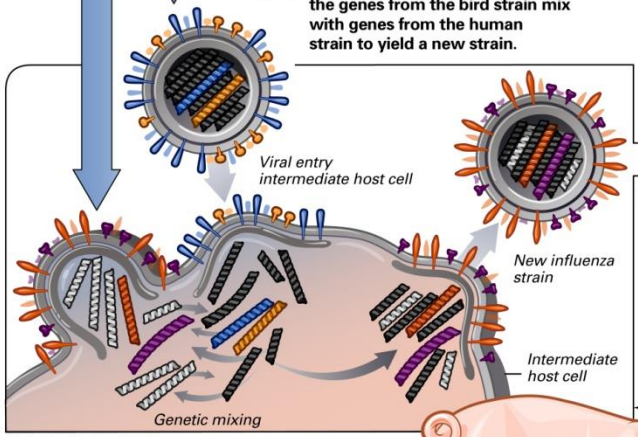


A-1 A duck or other aquatic bird passes a bird strain of influenza A to an intermediate host such as a chicken or pig.

A-2 A person passes a human strain of influenza A to the same chicken or pig. *(Note that reassortment can occur in a person who is infected with two flu strains.)*

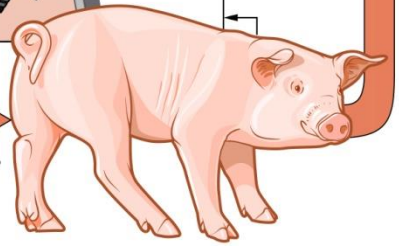
A-3 When the viruses infect the same cell, the genes from the bird strain mix with genes from the human strain to yield a new strain.

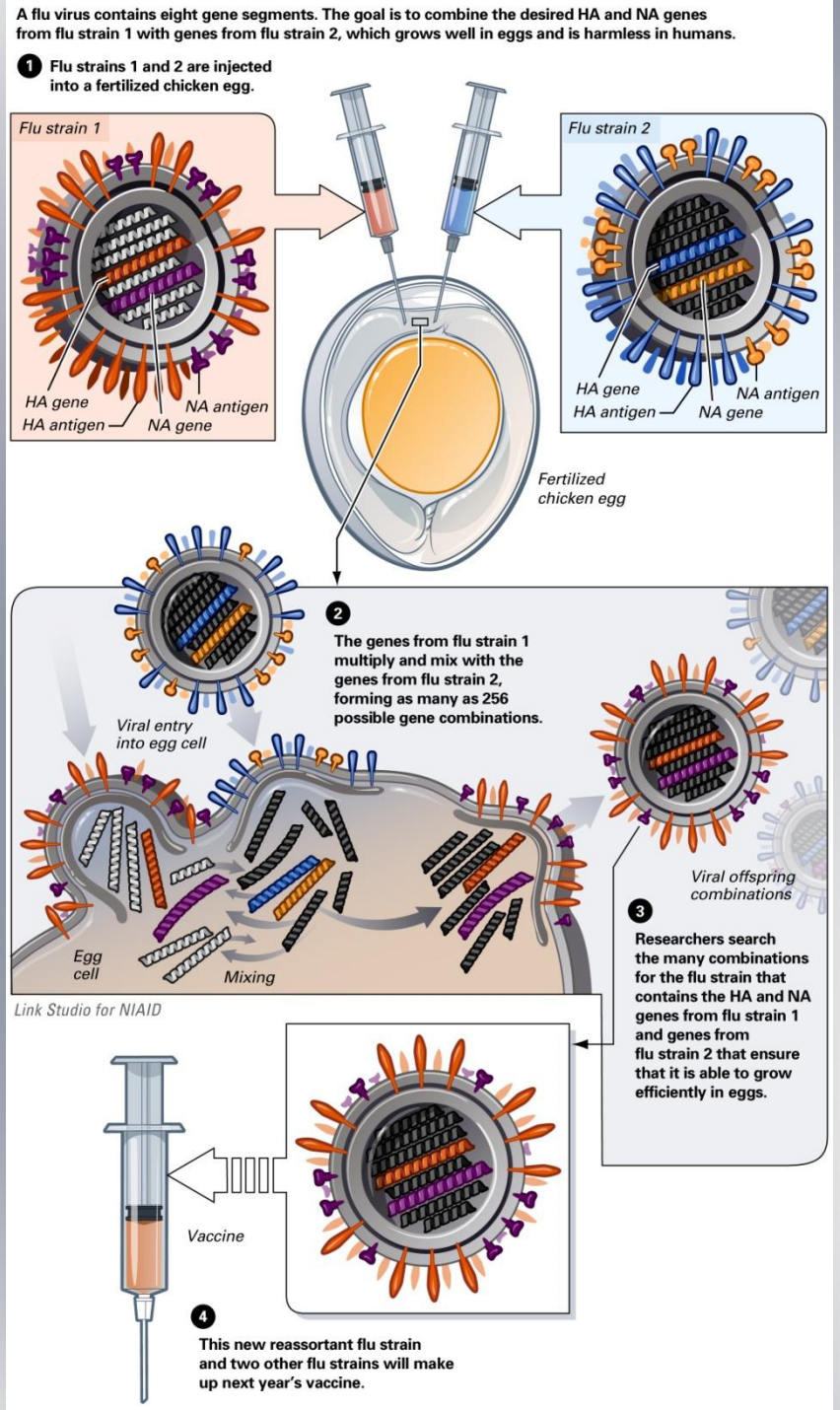
C Without undergoing genetic change, a bird strain of influenza A can jump directly from a duck or other aquatic bird to an intermediate animal host and then to humans.



A-4 The new strain can spread from the intermediate host to humans.

Link Studio for NIAID





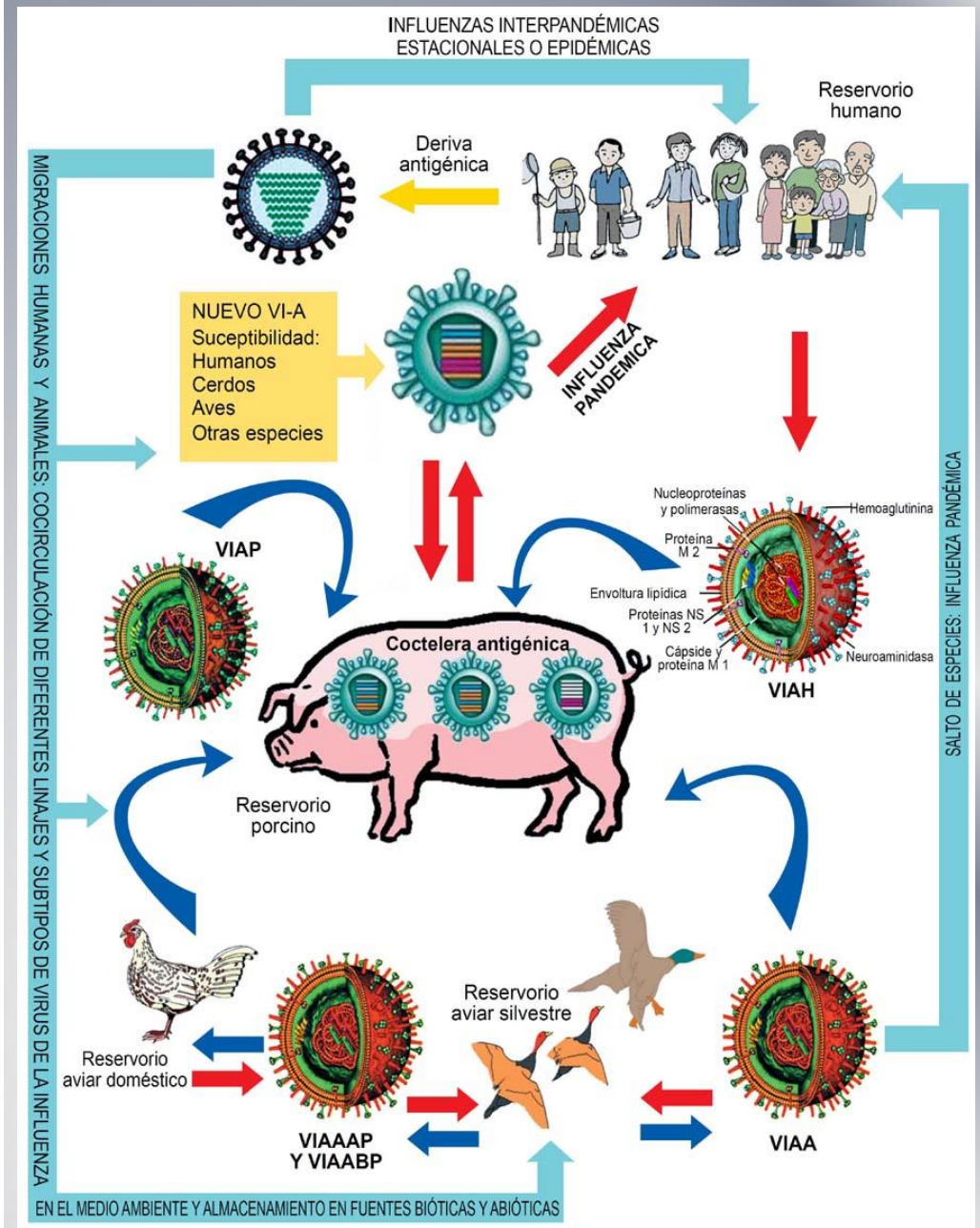


Figura 1. Los reservorios de los VI-A del linaje humano, aviar y porcino, interaccionan en un ecosistema o medioambiente común. En estas condiciones los VI-A siguen caminos de derivas antigénicas o cambios antigénicos menores, así como de reordenamientos o cambios antigénicos mayores. En el caso de los reordenamientos de los VI-A, el cerdo posee receptores para los VIAA y VIAH, por consiguiente puede infectarse simultánemente con ambos linajes y el suyo mismo sirviendo de una coctelera antigénica. En ciertas oportunidades los VI-A pueden saltar directamente de una especie a otra como lo observado con el VIAA H5N1

Pandemias de Influenza del siglo XX

Credit: US National Museum of Health and Medicine

1918: "Gripe Española" A(H1N1)

20-40 millones de muertos

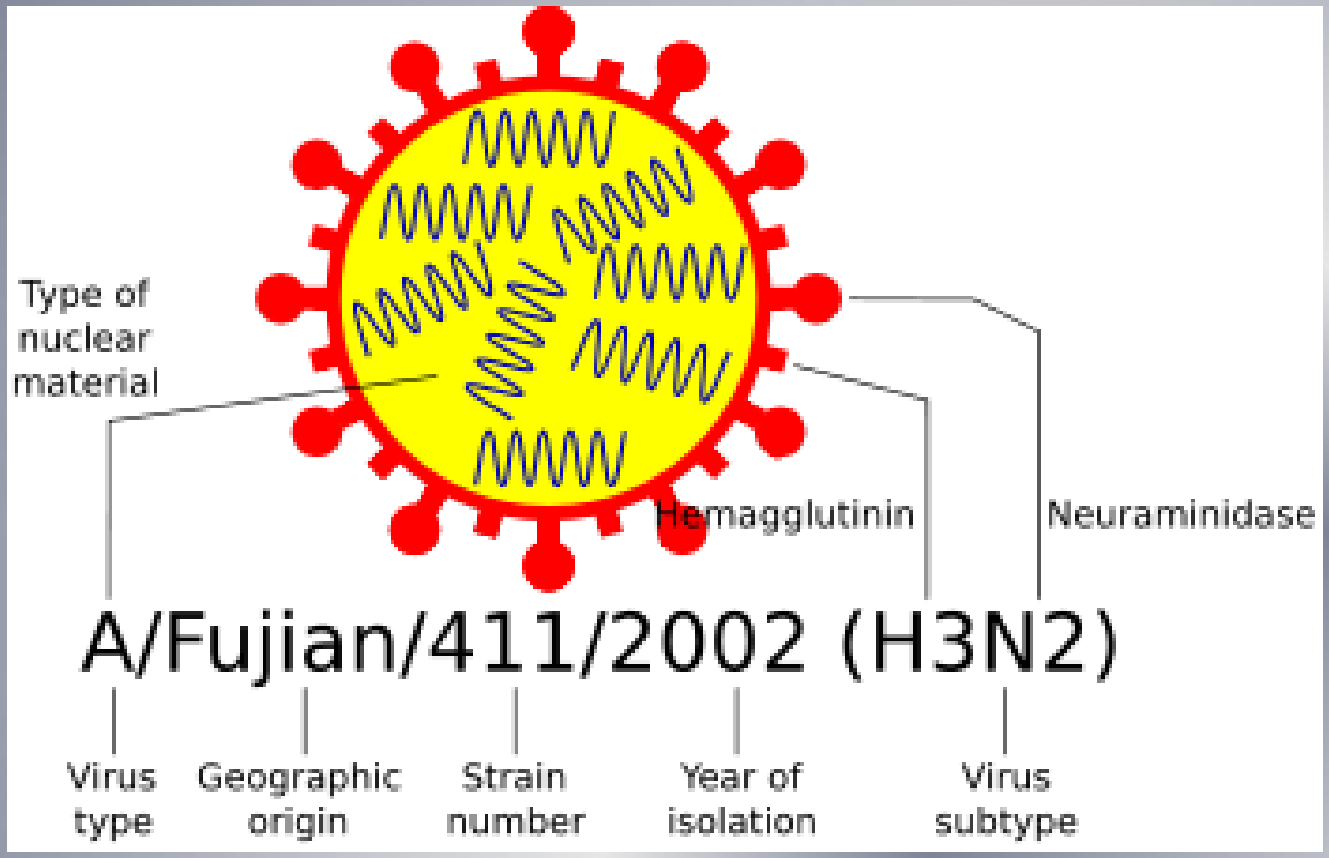
1957: "Gripe Asiática" A(H2N2)

1-4 millones de muertos

1968: "Gripe de Hong Kong" A(H3N2)

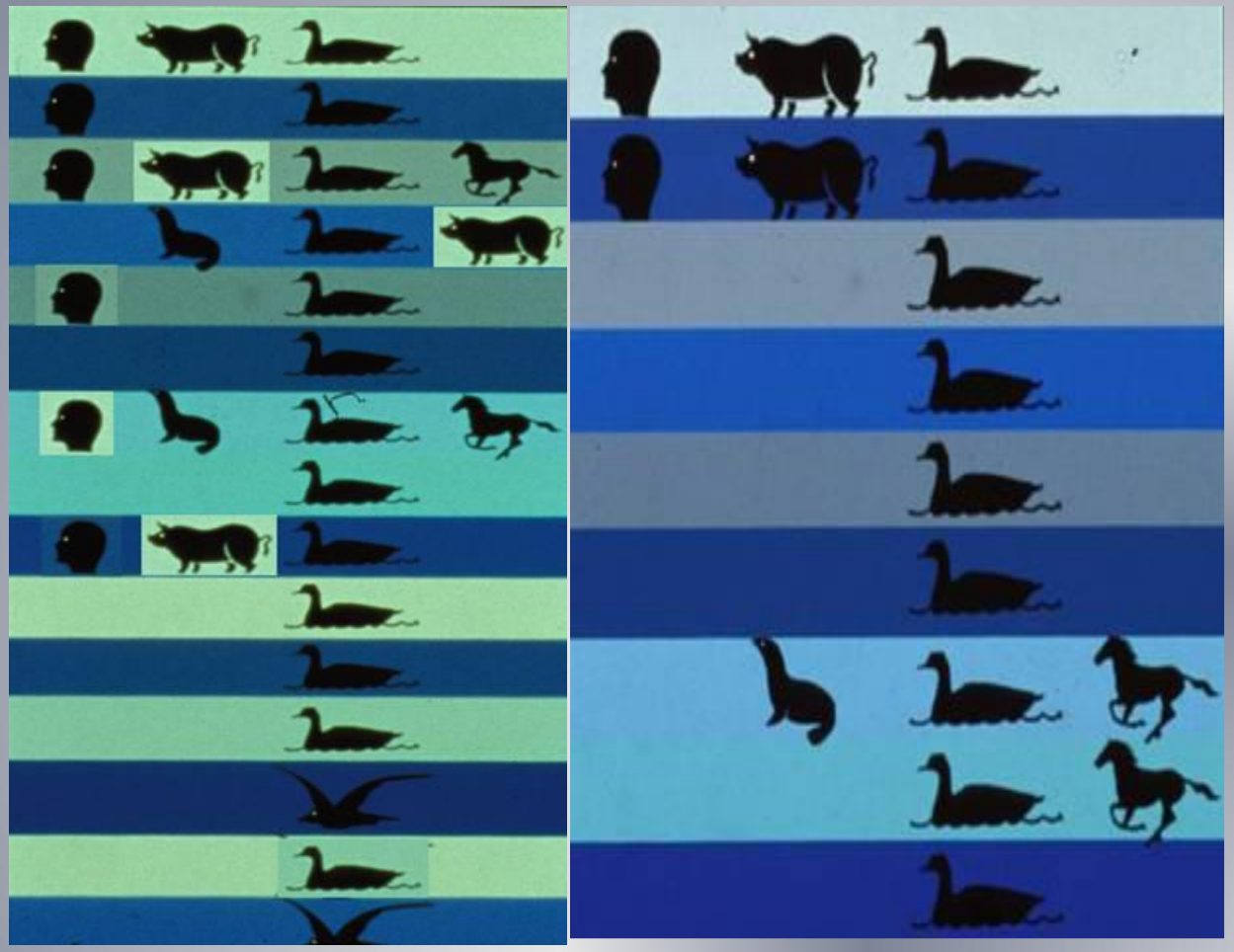
1-4 millones de muertos

Nomenclatura de Influenza A



Subtipos HA y NA en virus Influenza A

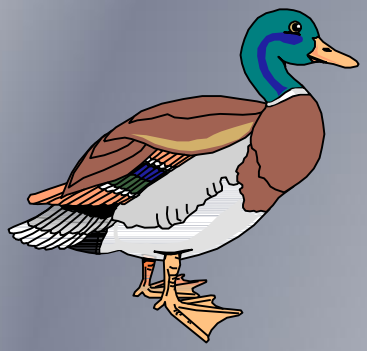
H1
H2
H3
H4
H5
H6
H7
H8
H9
H10
H11
H12
H13
H14
H15, 16



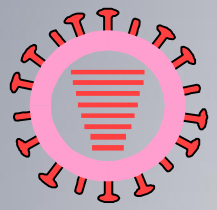
N1
N2
N3
N4
N5
N6
N7
N8
N9

Subtipos : **HXNX**

EMERGENCIA DE VIRUS PANDEMICO

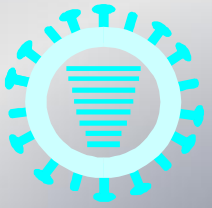


16HAs
9 NAs

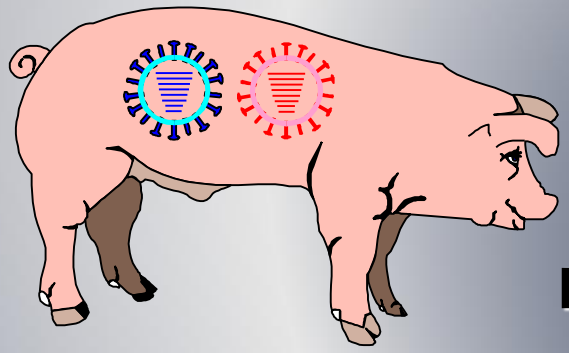


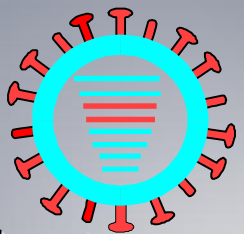
Virus Aviar

DIRECTO



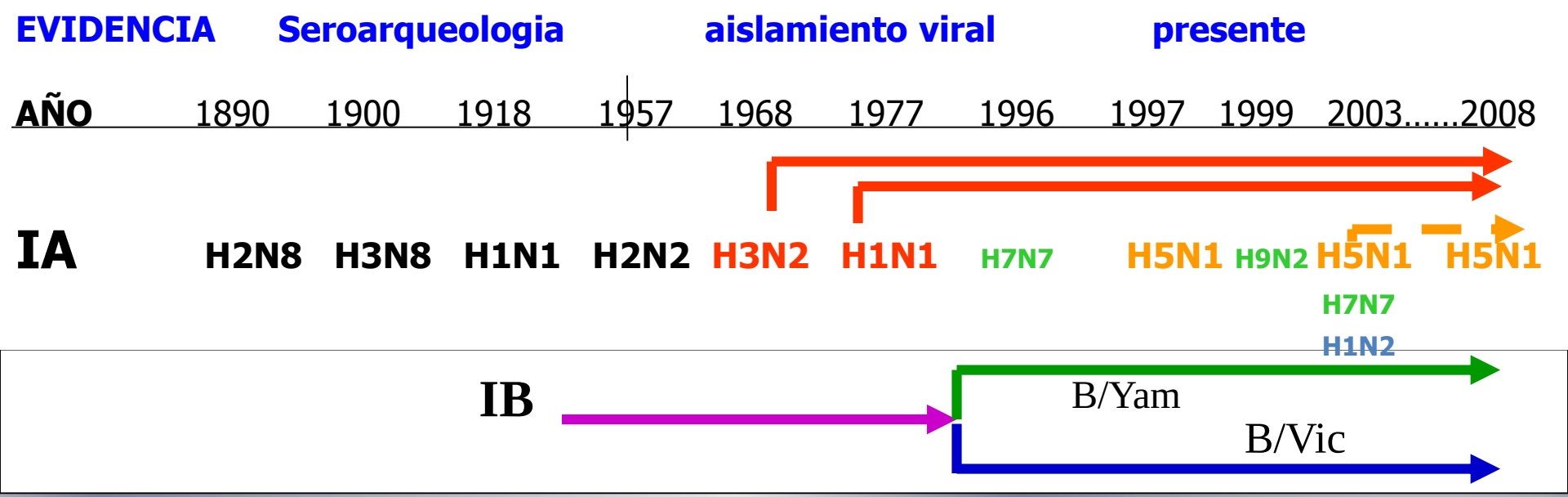
Virus
Humano





Reordenamiento
viral

CIRCULACION VIRUS INFLUENZA



¿Porque el subtipo H5N1 es Preocupante?

- **Muta rapidamente y de alta patogenicidad**
- **Causa enfermedad grave en humanos (casos Hong Kong, Viet Nam, Tailandia)**
- **Las aves que sobreviven a la infección excretan virus durante más de 10 días.**
- **La propagación de la infección entre las aves aumenta la probabilidad de una infección directa al hombre**
- **Si aumenta el n° de personas infectadas con H5N1, aumenta la probabilidad de reordenamientos con cepas gripales humanas, facilitando la transmisión persona-persona**

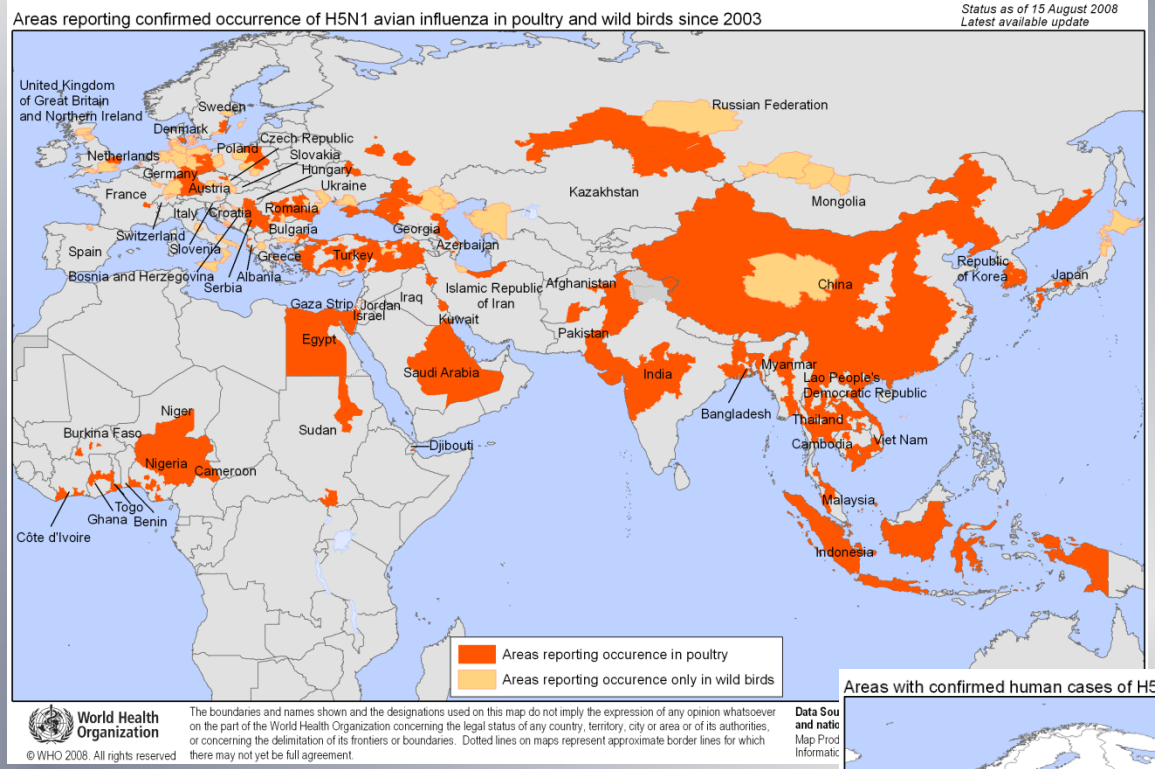
H5N1 EN HUMANOS

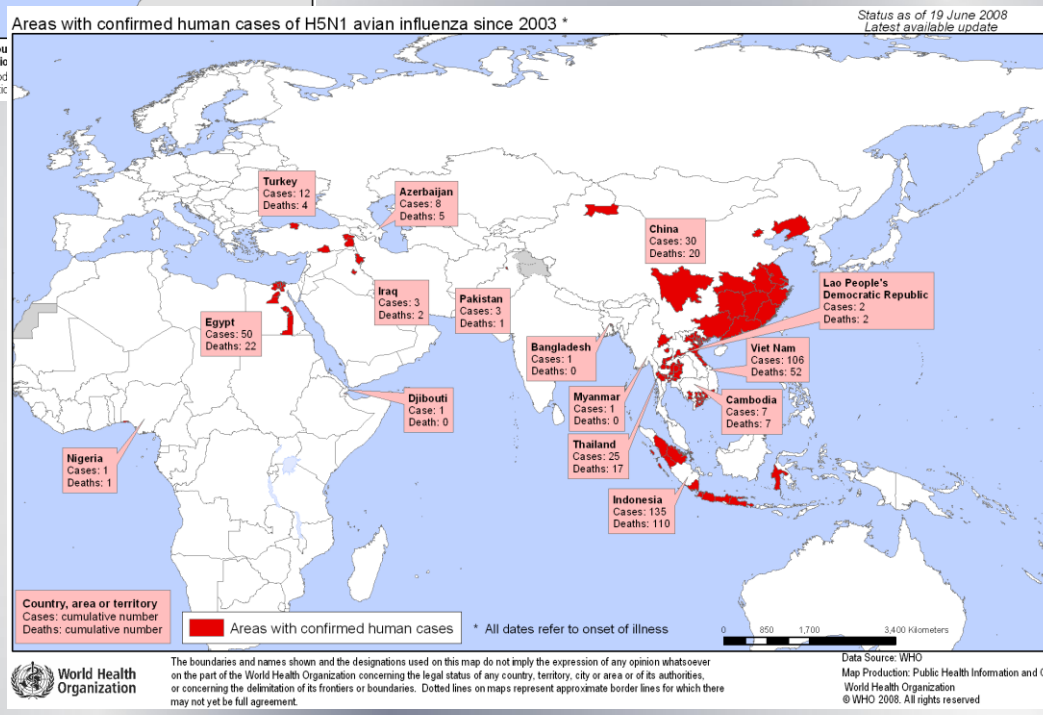
328 casos humanos

200 muertos

12 países afectados

OMS, 9/2007





¿Pueden evitarse las pandemias de Gripe?

- **Las pandemias de gripe aparecen 3 o 4 veces cada siglo**
- **La aparición de una Pandemia de Gripe es impredecible**
- **Según los últimos informes de la OMS la pandemia de gripe es inevitable y posiblemente inminente.**

Medidas para reducir los riesgos:

- ✓ **Detener la propagación de la epidemia en población de aves de corral y silvestre, reduciendo la exposición humana al virus**
- ✓ **Vacunación de las personas con alto riesgo de exposición a aves infectadas , reduciendo probables reordenamientos genéticos**
- ✓ **Indumentaria y material adecuados en el personal que participa en la matanza de aves**
- ✓ **Tratamiento con antivirales como medida profiláctica en población expuesta y/o en zona de brote (amantadina, rimantadina, oseltanavir, zanamavir)**
- ✓ **Vigilancia Epidemiológica de virus circulantes en animales y humanos a nivel mundial**

RED DE VIGILANCIA MUNDIAL DE INFLUENZA

Y ADEMÀS….

Principales Virus Entéricos

Virus	Enfermedad
Adenovirus	Varias
Enterovirus	
Poliovirus	Poliomielitis
	Parálisis
Echovirus	Varias
Coxsackievirus	Varias
Hepatitis A	Hepatitis infecciosa
Reovirus	Varias
Rotavirus	Gastroenteritis

PATOLOGÍAS VÍRICAS

Virus	Enfermedad	Receptor
Adenovirus	Varias	Hombre
Poliovirus Enterovirus	Poliomelitis, parálisis y otras	Hombre
Coxsackievirus	Varias	Hombre
Hepatitis A	Hepatitis infecc.	Hombre
Reovirus	Varias	Hombre
Rotavirus	Diarrea	Hombre/ animales
Agentes Norwalk	Gastroenteritis agudas e infecciones no bacterianas	Hombre

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People.™

Healthcare Infection Control Practices Advisory Committee (HICPAC)

Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008

Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008 [PDF - 948 KB]

Figure 1. Decreasing order of resistance of microorganisms to disinfection and sterilization and the level of disinfection or sterilization.

Resistant	Level
Prions (Creutzfeldt-Jakob Disease)	Prion reprocessing
Bacterial spores (Bacillus atrophaeus)	Sterilization
Coccidia (Cryptosporidium)	
Mycobacteria (M. tuberculosis, M. terrae)	High
Nonlipid or small viruses (polio, coxsackie)	Intermediate
Fungi (Aspergillus, Candida)	
Vegetative bacteria (S. aureus, P. aeruginosa)	Low
↓ Lipid or medium-sized viruses (HIV, herpes, hepatitis B)	

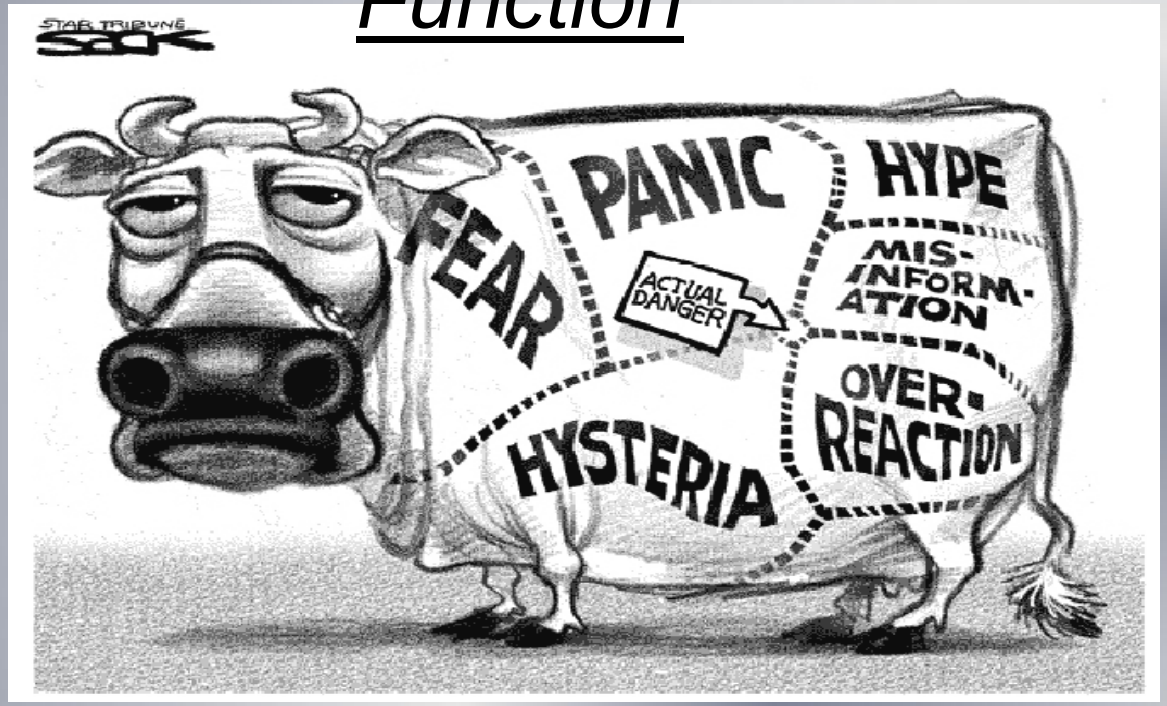
Susceptible

Modified from Russell and Favero 13, 344.

Next Table

How Now Mad Cow?

Introduction to Prion Disease and Function



SHP – Neurobiology of Development and Disease

¿QUÉ ES UN PRIÓN?

Los priones son partículas infecciosas proteicas las cuales resisten la inactivación por medio de procedimientos a los cuales los ácidos nucleicos son sensibles.

La proteina priónica (PrPsc,) causante de la enfermedad es una forma modificada de una proteína normal llamada PrPc sensible a proteasas, por su parte la PrPsc es resistente a las proteasas.

La ingesta de PrPsc induce la conversión de PrPc en PrPsc, lo cual lleva a la propagación de la forma infecciosas y a la aparición de la enfermedad.

Progression of BSE

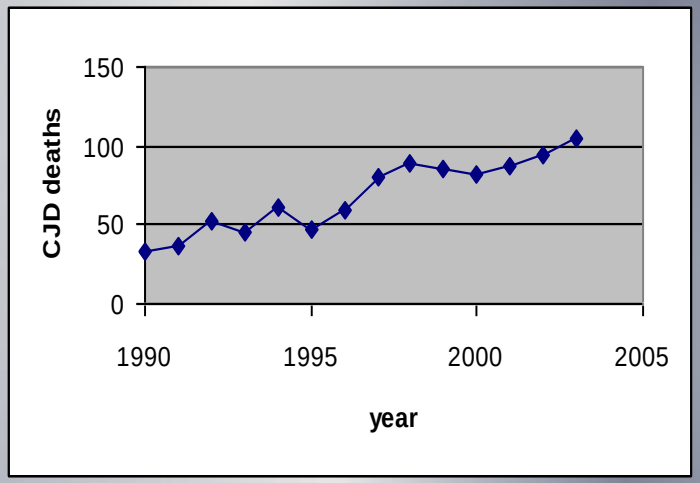
- 1986: First case of BSE discovered in a cow that was fed livestock feed produced from a sheep that died of scrapie.
- Dr. Richard Lacey annouces that scrapie and BSE are the same disease and that “this beef was in the meat supply”.
- British government dismisses Lacey and cuts his research funding. They announce that scrapie renderings are still an acceptable form of livestock feed.

Progression of BSE (cont)

- 1987: 700 BSE infected cows are reported in Great Britain.
- 1988: 7,000 infected cows. Law is passed declaring sheep rendering illegal.
- 1992: 36,000 infected livestock reported.
- 1994: 150,000 infected livestock reported and is identified in half of British cattle herds.

Crossing the line…..

- In 1996, a new form of CJD is discovered in the UK, termed variant CJD (vCJD).
- Linked with consumption of BSE-contaminated beef.
- Shares the symptoms of classic CJD, except the median age of death is 28 (contrasting with 68) and feature psychiatric and sensory symptoms with neurologic effects occuring later.

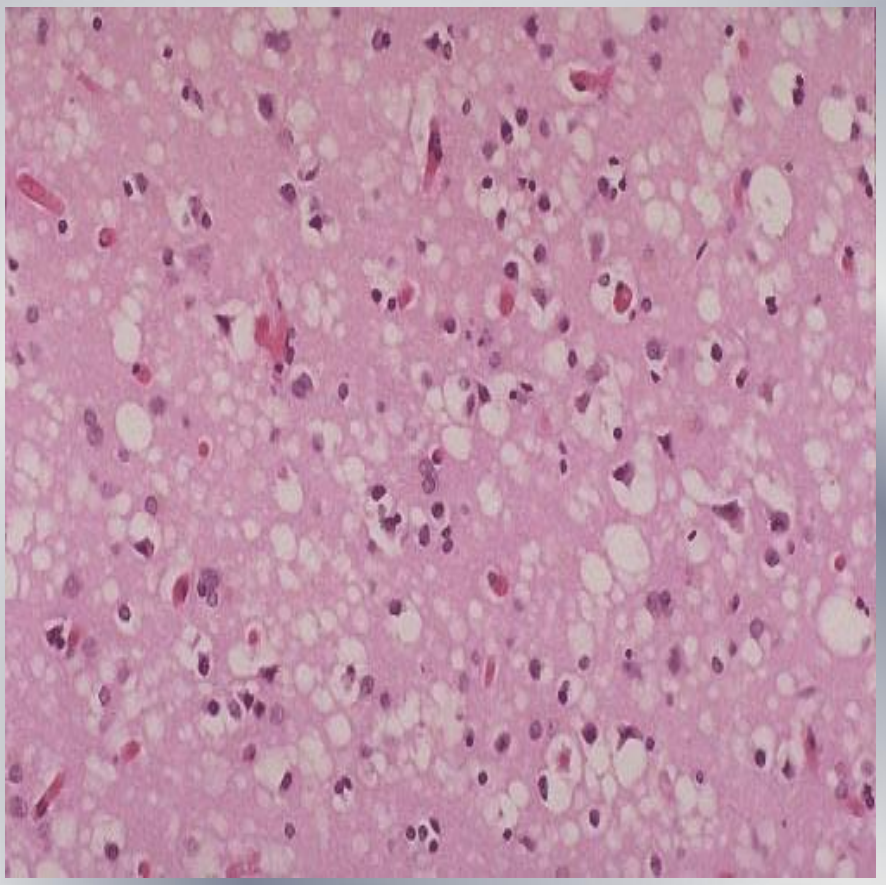


EET (TSE) EN HUMANOS

- Creutzfeldt-Jakob Disease (CJD)
- Variant Creutzfeldt-Jakob Disease (vCJD)
- Gerstmann-Straussler-Scheinker Syndrome
- Fatal Familial Insomnia
- Kuru

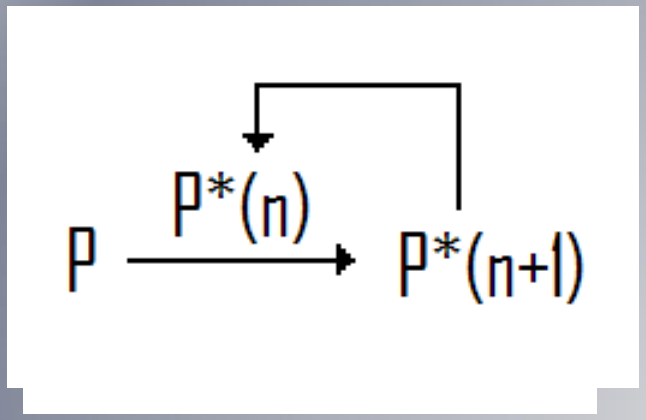
ENCEFALOPATÍAS ESPONGIFORMES TRANSMISIBLES (EET ó TSE)

- **Constituyen una familia de enfermedades neurodegenerativas, de "baja incidencia", que afectan tanto a los animales como a los humanos.**
- **Se caracterizan por su largo periodo de incubación, grandes cambios (pérdida) de la masa neuronal (aparición de agujeros, como esponjas) y la incapacidad del sistema inmunológico de producir una respuesta inflamatoria.**

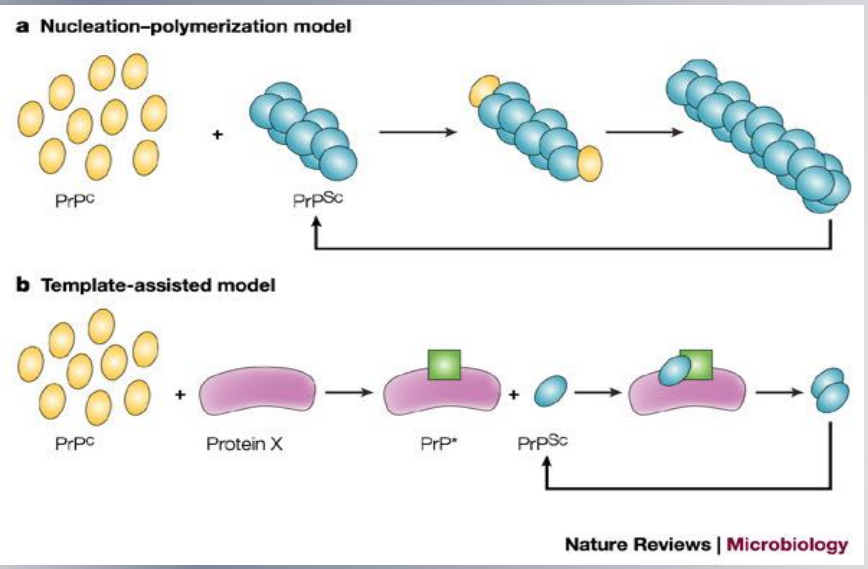


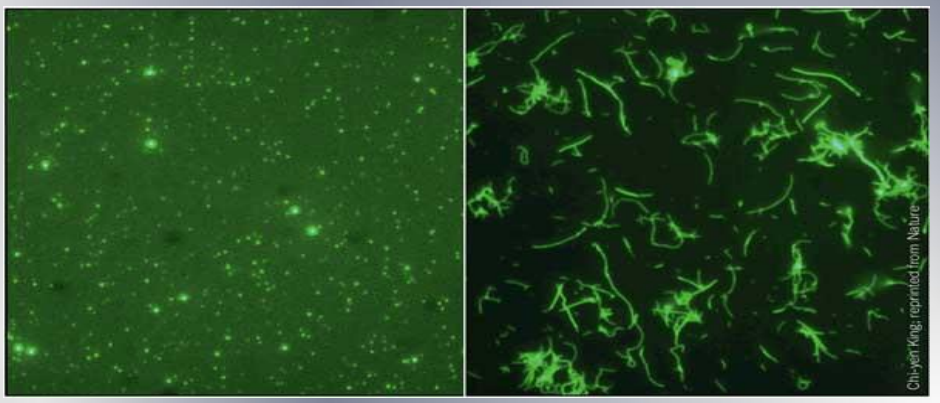
- **INFECCIOSO / CONTAGIO EXÓGENO**
  - Ejemplos en Kuru, BSE (enfermedad de la vaca loca), Scrapie
  - Diseminada por:
    - Consumo de carne infectada.
    - Transfusión
- **ESPORÁDICO**
- **FAMILIAR / HEREDITARIO**
  - Debido a una mutación autosómica dominante de *prP*?

Molecular Mechanism



- **Protein can convert between two conformations (a benign form and pathogenic state) at a certain frequency.**
- **The second state can seed the formation of oligometric, insoluble aggregates that in turn form toxic amyloid plaques.**
- **During the oligomerization the prions corrupt the native form of the protein into a transmissible disease conformation.**





Funciones de la proteína priónica normal

- Antioxidante

PrP^C + Cu (Cobre)

↓

Actividad antioxidante

↓

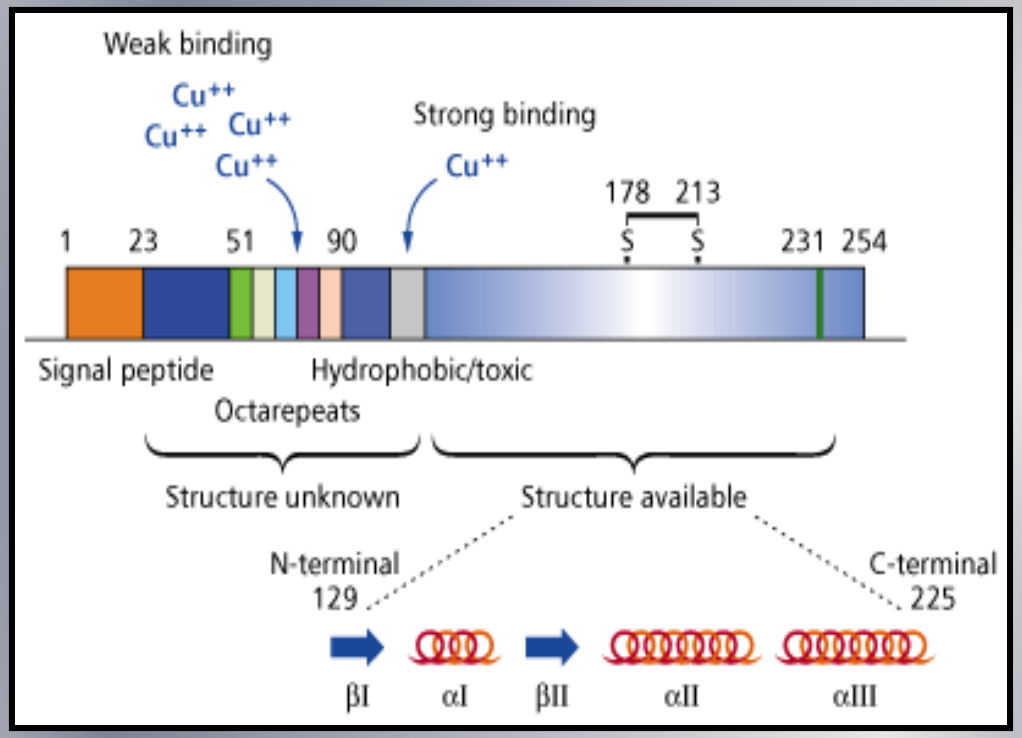
Resistencia al estrés oxidativo

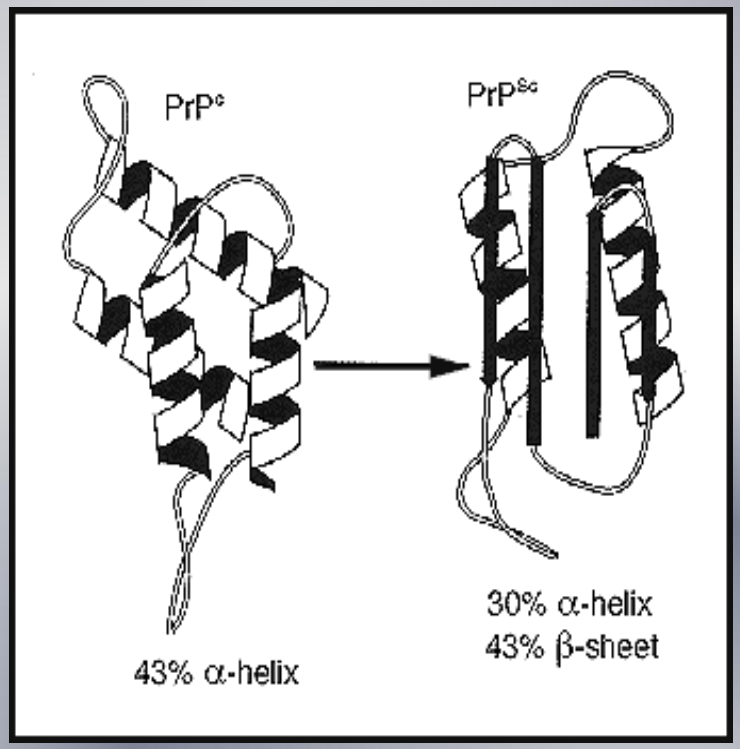
↓

Prevención de la disfunción neuronal

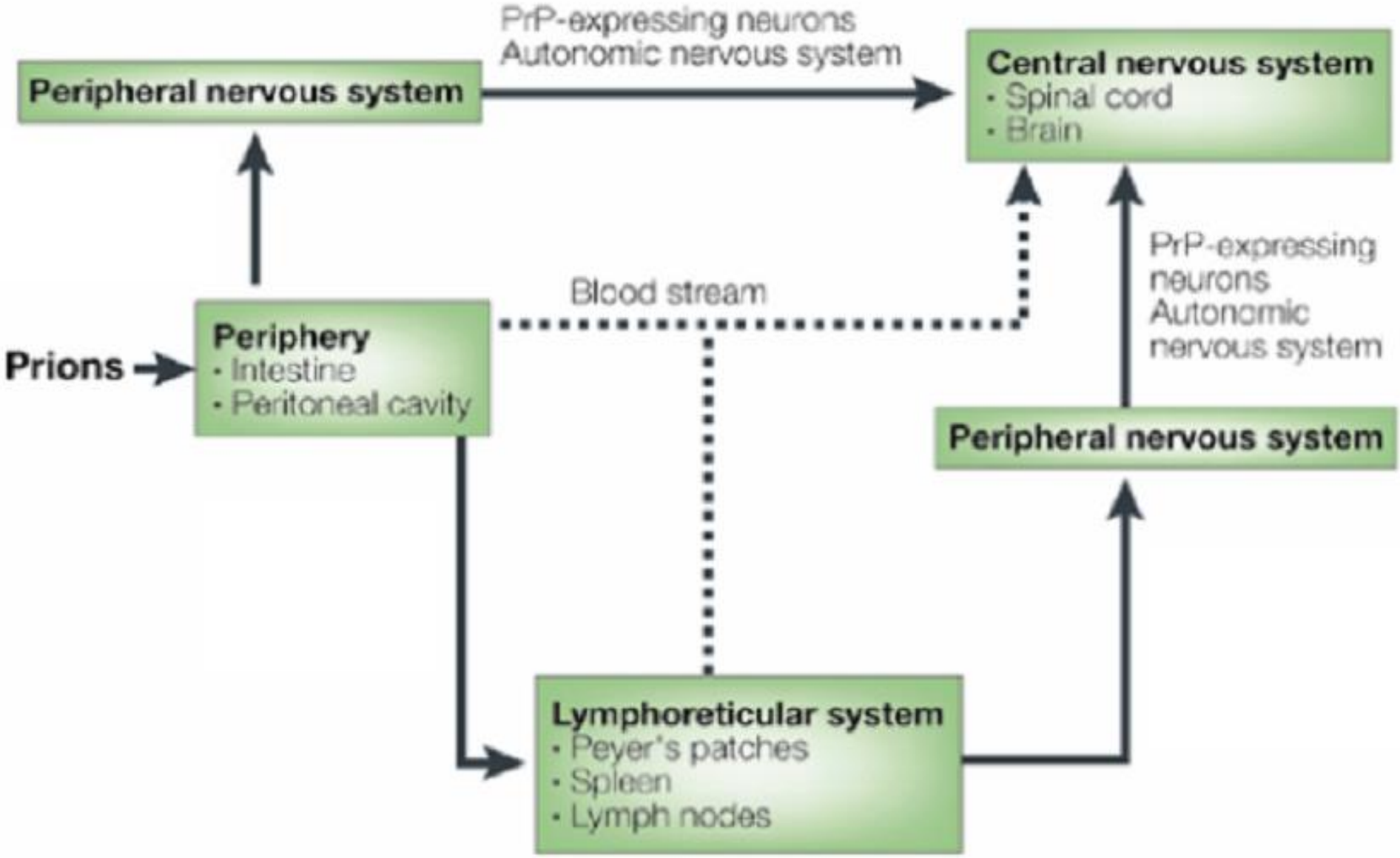
- Otras funciones?

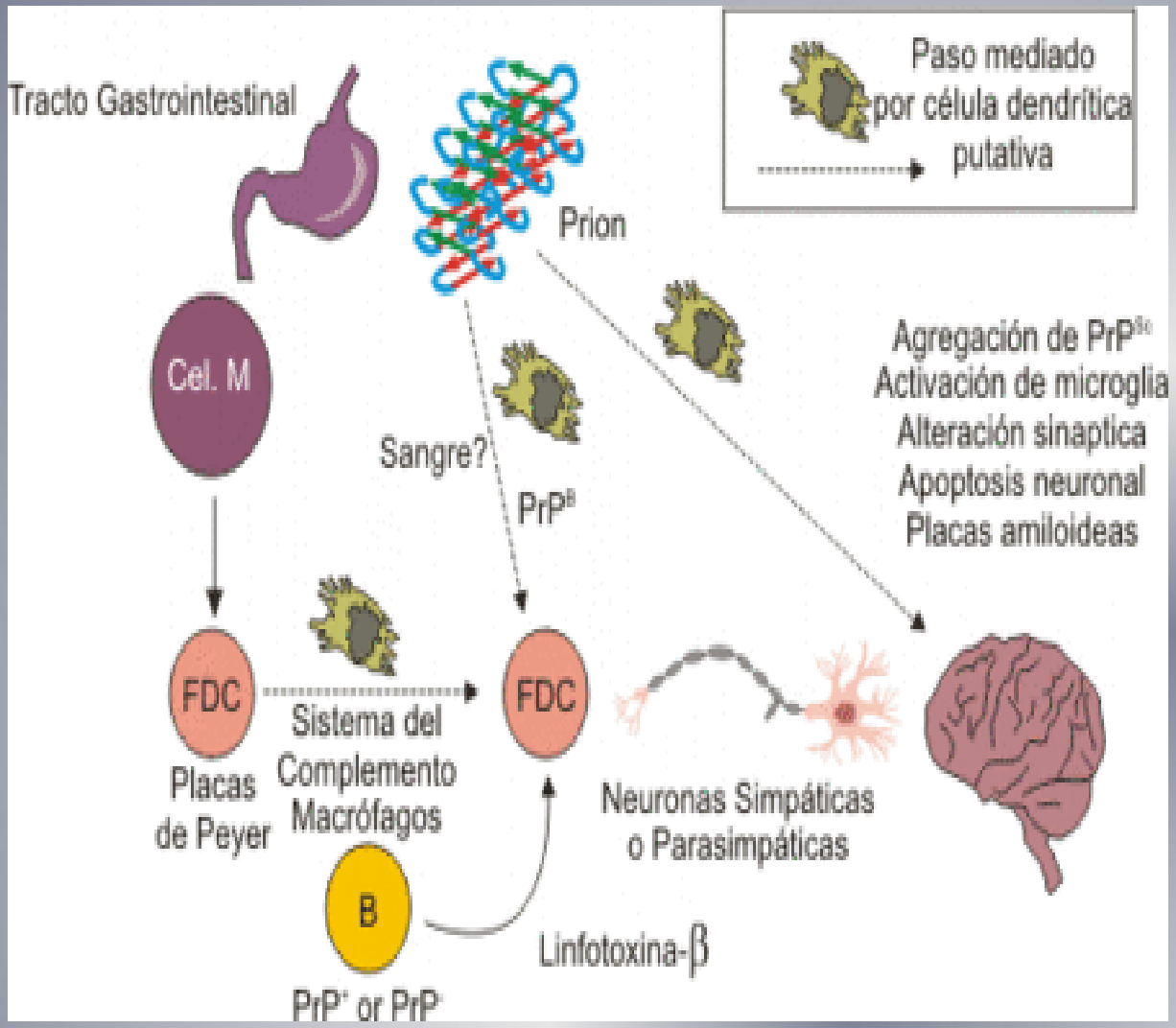
Sequence of prion protein



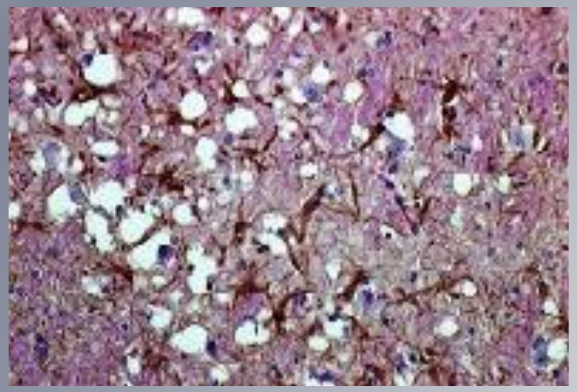


Pathogenesis

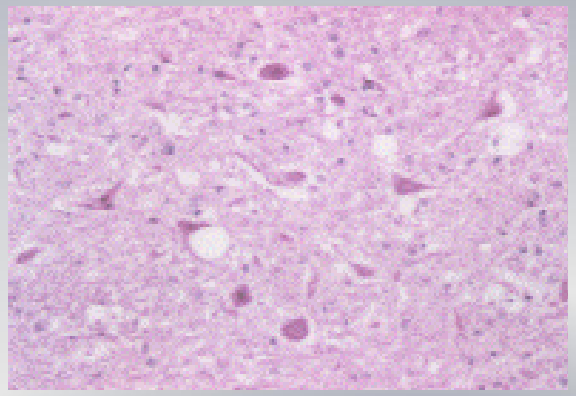



Paso mediado por célula dendrítica putativa
Tracto Gastrointestinal
Prion
Cel. M
Sangre?
PrP^Sc
Agregación de PrP^Sc
Activación de microglia
Alteración sinaptica
Apoptosis neuronal
Placas amiloideas
FDC
Placas de Peyer
Sistema del Complemento Macrófagos
FDC
Neuronas Simpáticas o Parasimpáticas
B
PrP+ or PrP-
Linfotoxina-β

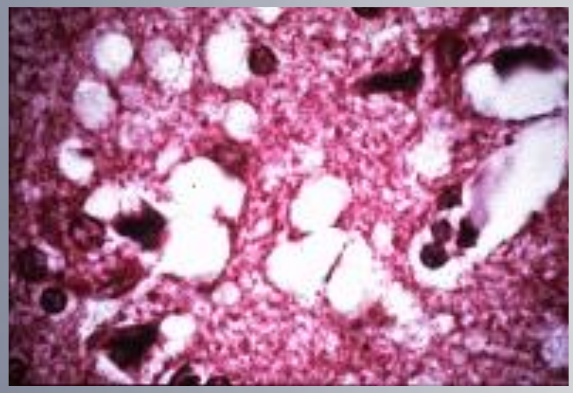
Microscopic changes



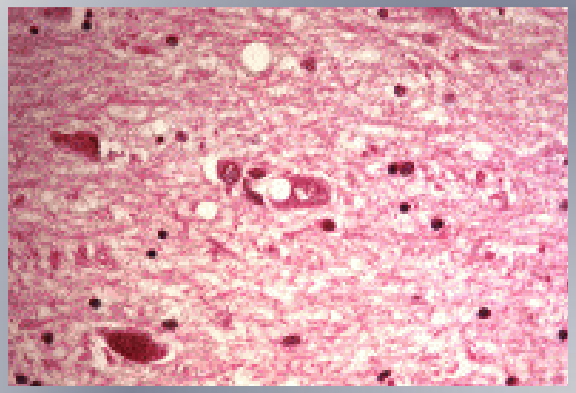
Scrapie



BSE



Kuru



CJD

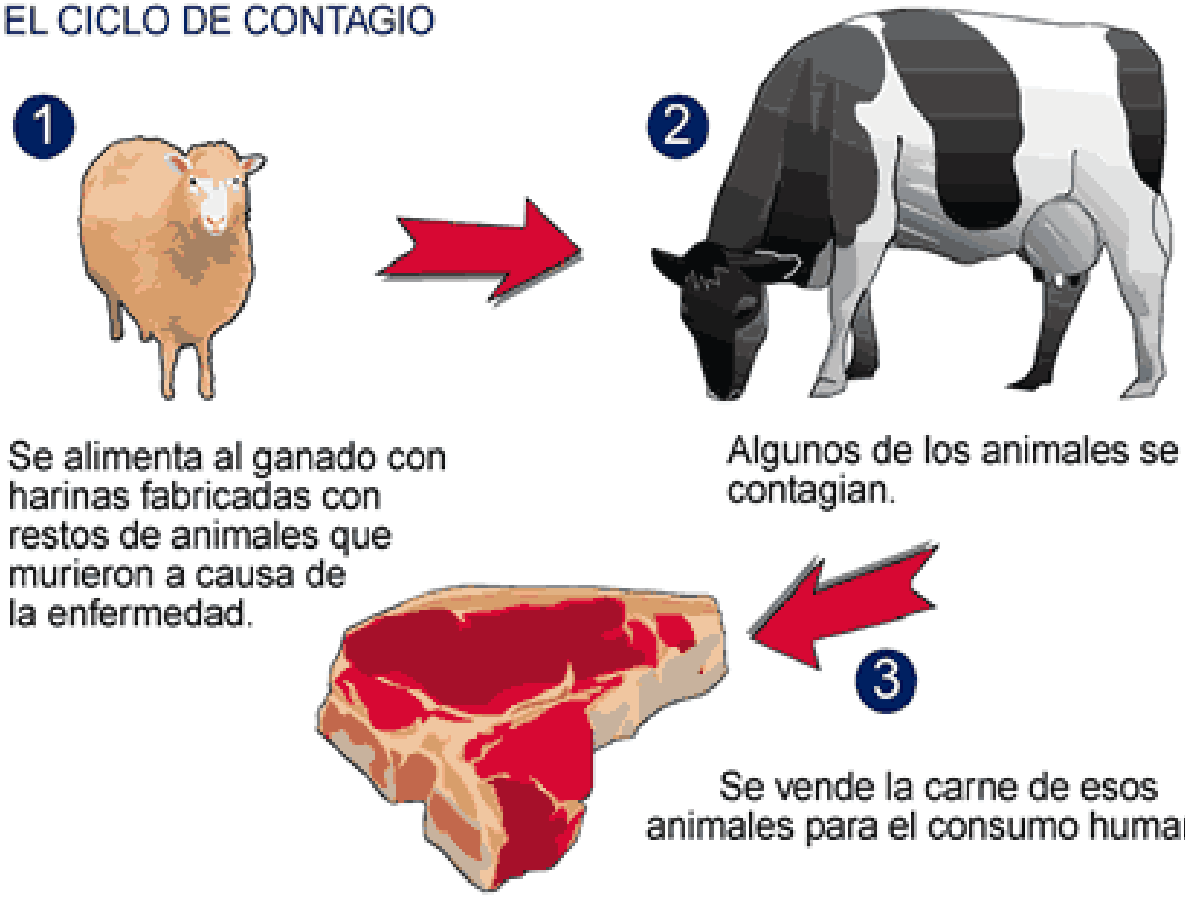
La enfermedad de las vacas locas

La encefalopatía espongiforme bovina (EEB) es una enfermedad mortal que se puede contraer por el consumo de carne de animales enfermos.

¿Qué la origina?

La EEB es una alteración de unas proteínas situadas en el sistema nervioso central de los mamíferos llamadas proteínas priónicas.
Cuando se convierten en priones, proteínas con capacidad de autogenerarse e infectar células, comienzan a destruir el cerebro hasta la muerte del animal .

EL CICLO DE CONTAGIO



EFE/Puleva Salud

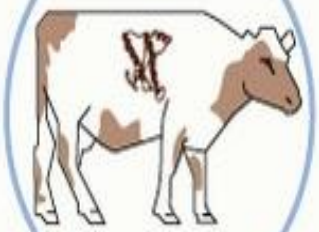
COMO SE TRANSMITE

El prion es una proteína alterada causante de un grupo de enfermedades degenerativas transmitidas al hombre por la ingestión de carne contaminada.

PROCESO

Una vaca puede infectarse con un solo gramo de alimento con priones

Los animales Infectados sufren espasmos y calambres que los hacen comportarse como trastornados



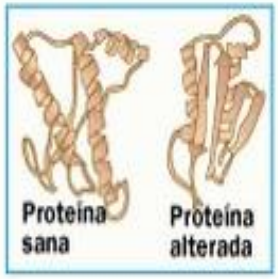
La carne y productos cárnicos de un animal contaminado lo transmite al hombre

CONTAGIO

Los priones degeneran y contagian a otras proteinas que las rodean produciendo agujeros en el cerebro

- Pérdida del habla

- Destrucción del sistema nervioso



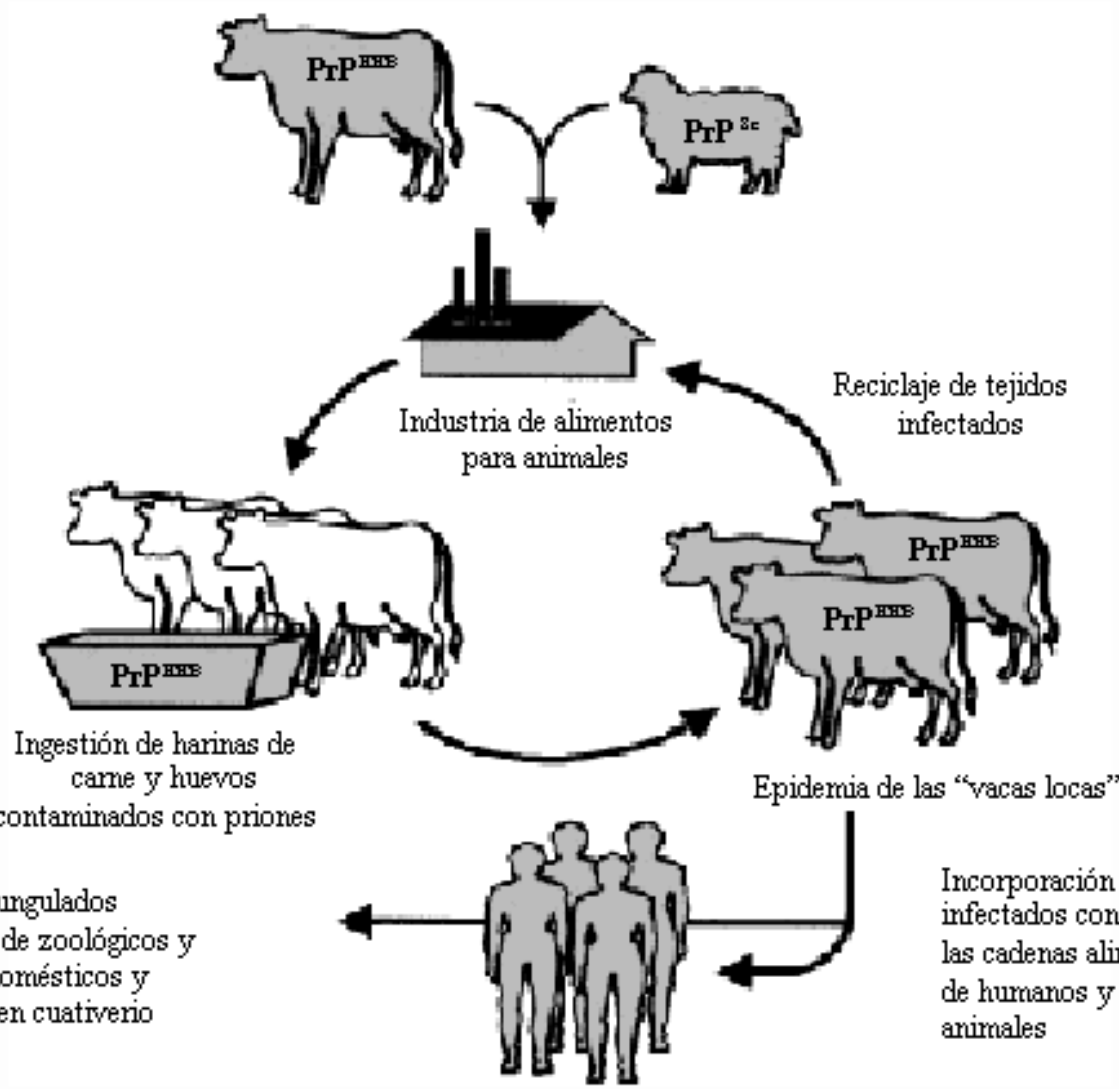
OTROS SINTOMAS

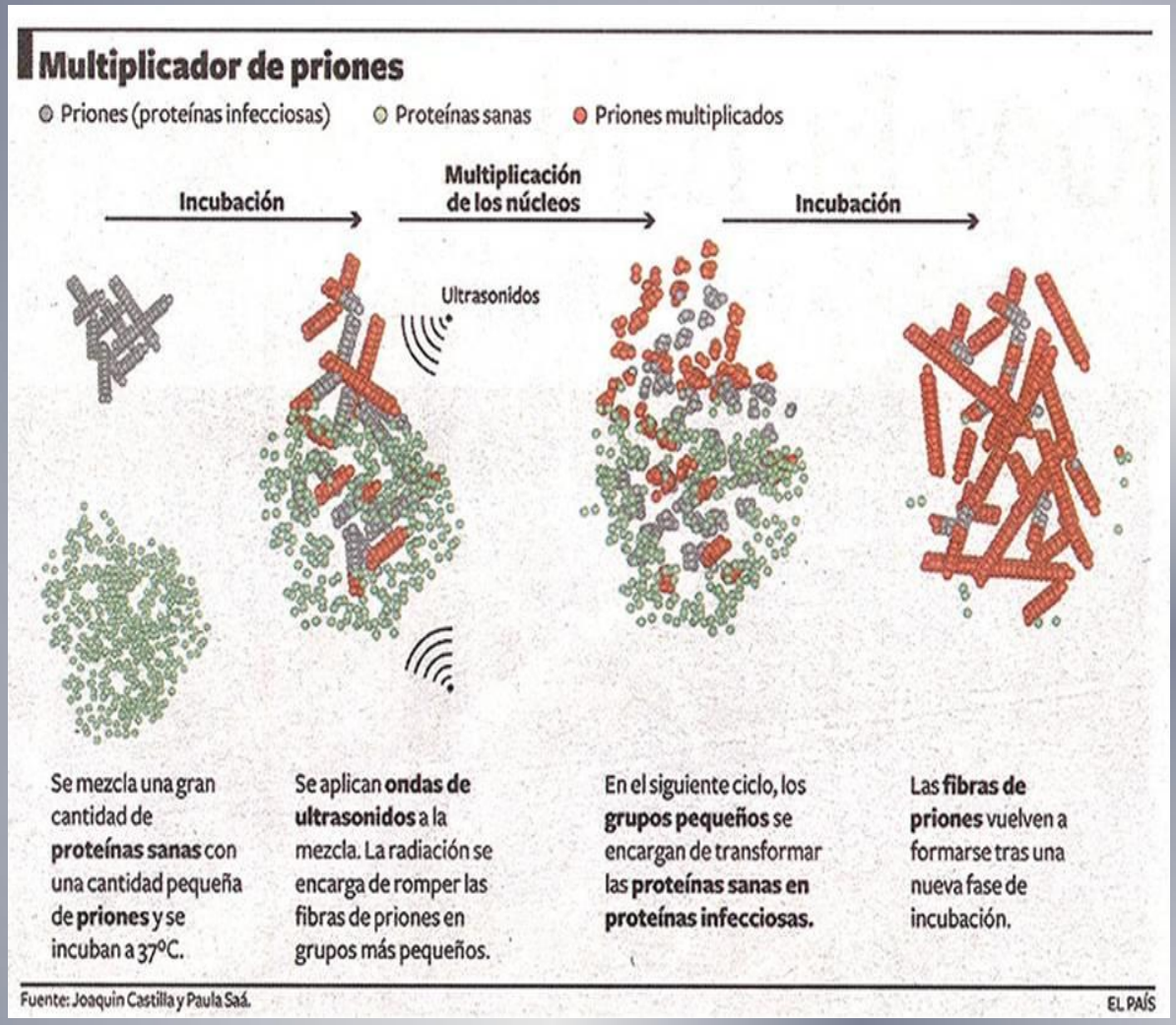
- Depresión y ansiedad
- Pérdida de la memoria, la visión, y el peso

Origen de la epidemia de EEB en el Reino Unido

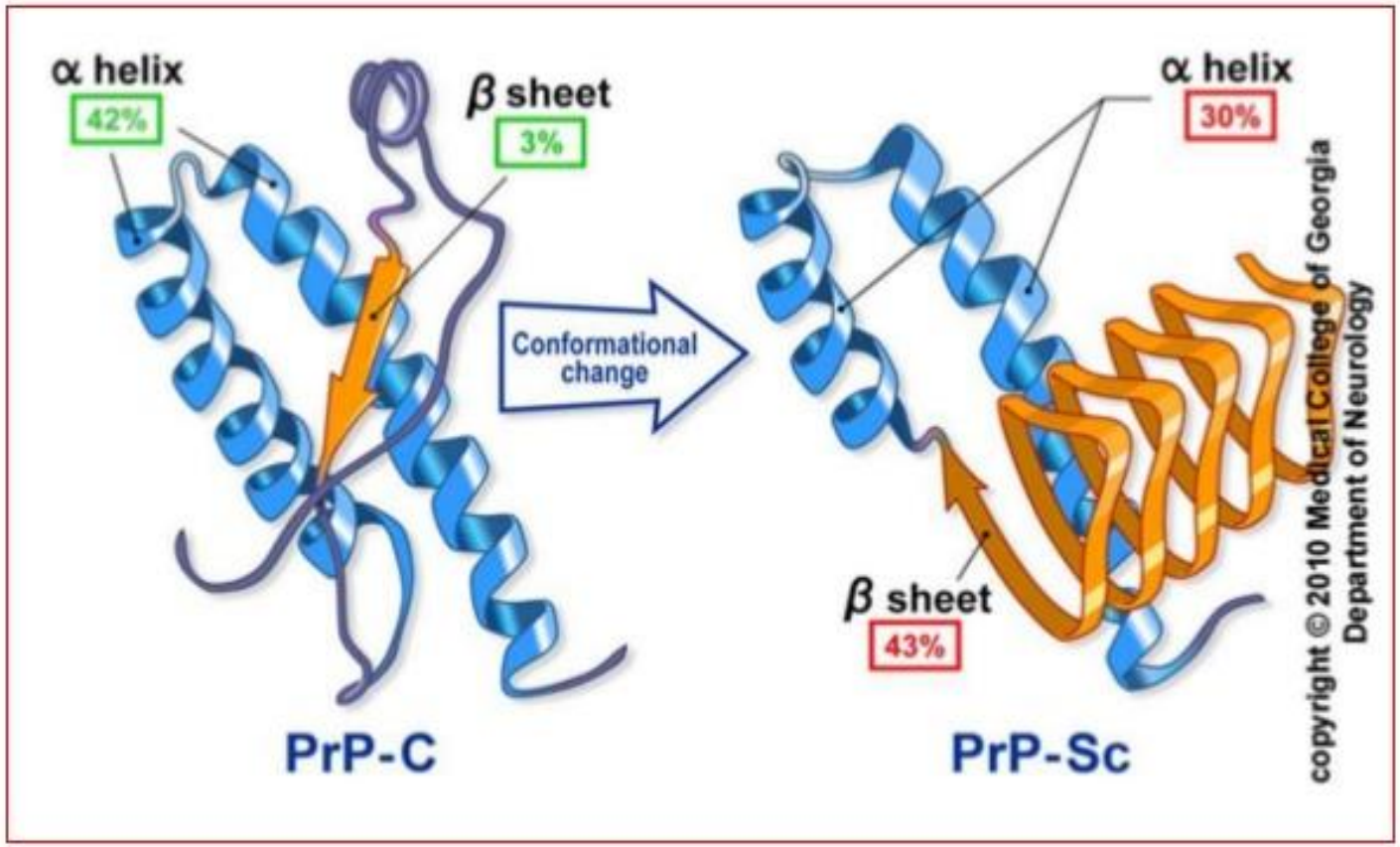
HIPÓTESIS

1. Existencia de **EEB** endémica previa a la epidemia
2. Mutación *de novo* en el gen de **PrP** de la vaca
3. Transmisión a partir del *scrapie* de las ovejas

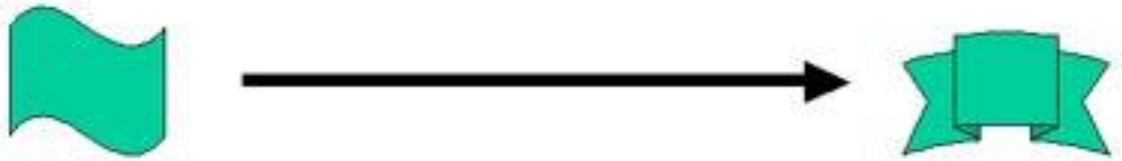



Multiplicador de priones
Priones (proteínas infecciosas)
Proteínas sanas
Priones multiplicados
Incubación
Multiplicación de los núcleos
Incubación
Ultrasonidos
Se mezcla una gran cantidad de proteínas sanas con una cantidad pequeña de priones y se incuban a 37ºC.
Se aplican ondas de ultrasonidos a la mezcla. La radiación se encarga de romper las fibras de priones en grupos más pequeños.
En el siguiente ciclo, los grupos pequeños se encargan de transformar las proteínas sanas en proteínas infecciosas.
Las fibras de priones vuelven a formarse tras una nueva fase de incubación.
Fuente: Joaquín Castilla y Paula Saá.
EL PAÍS

ESTRUCTURA PrPc y PrPSc



PRION PROTEIN (PrP)

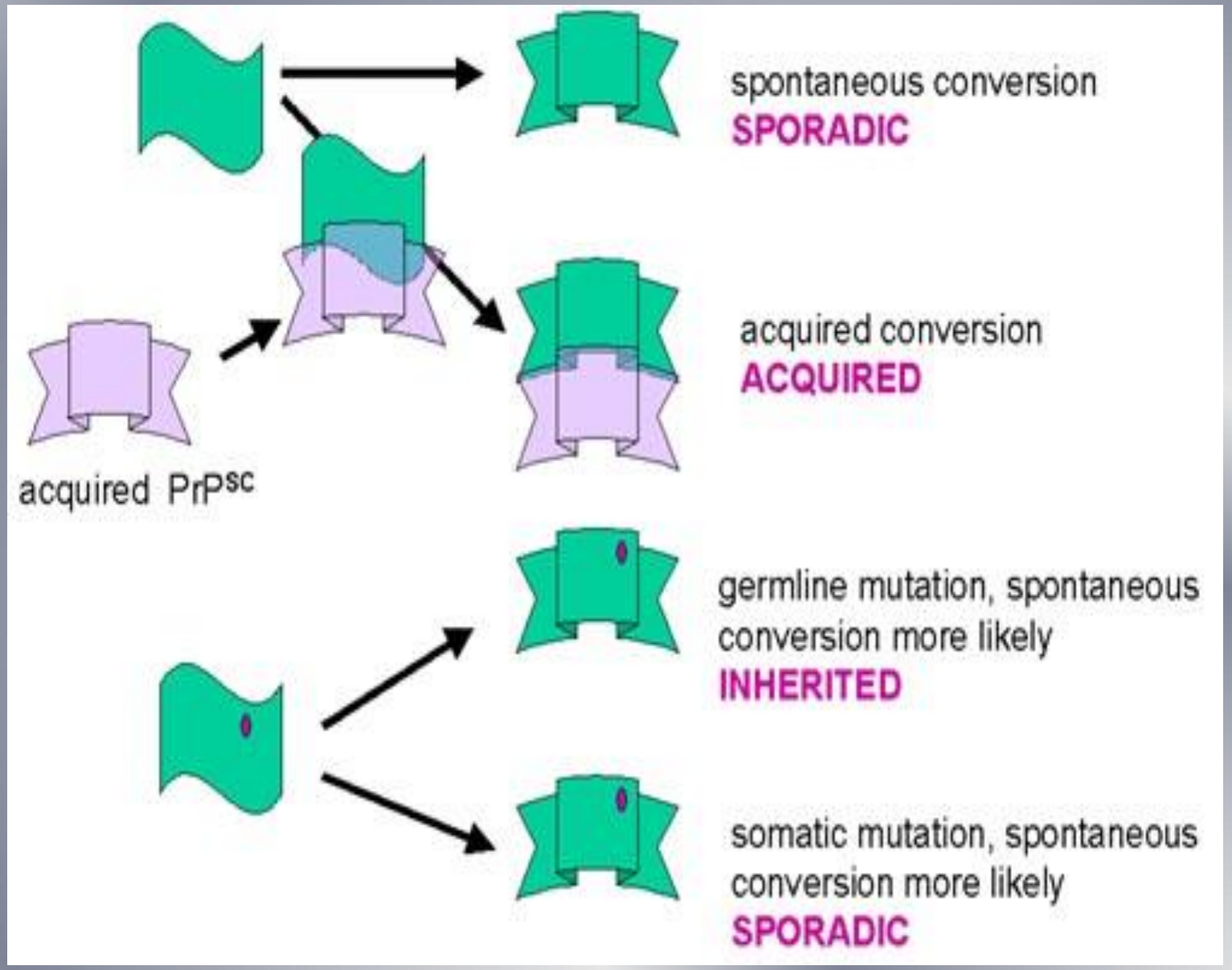


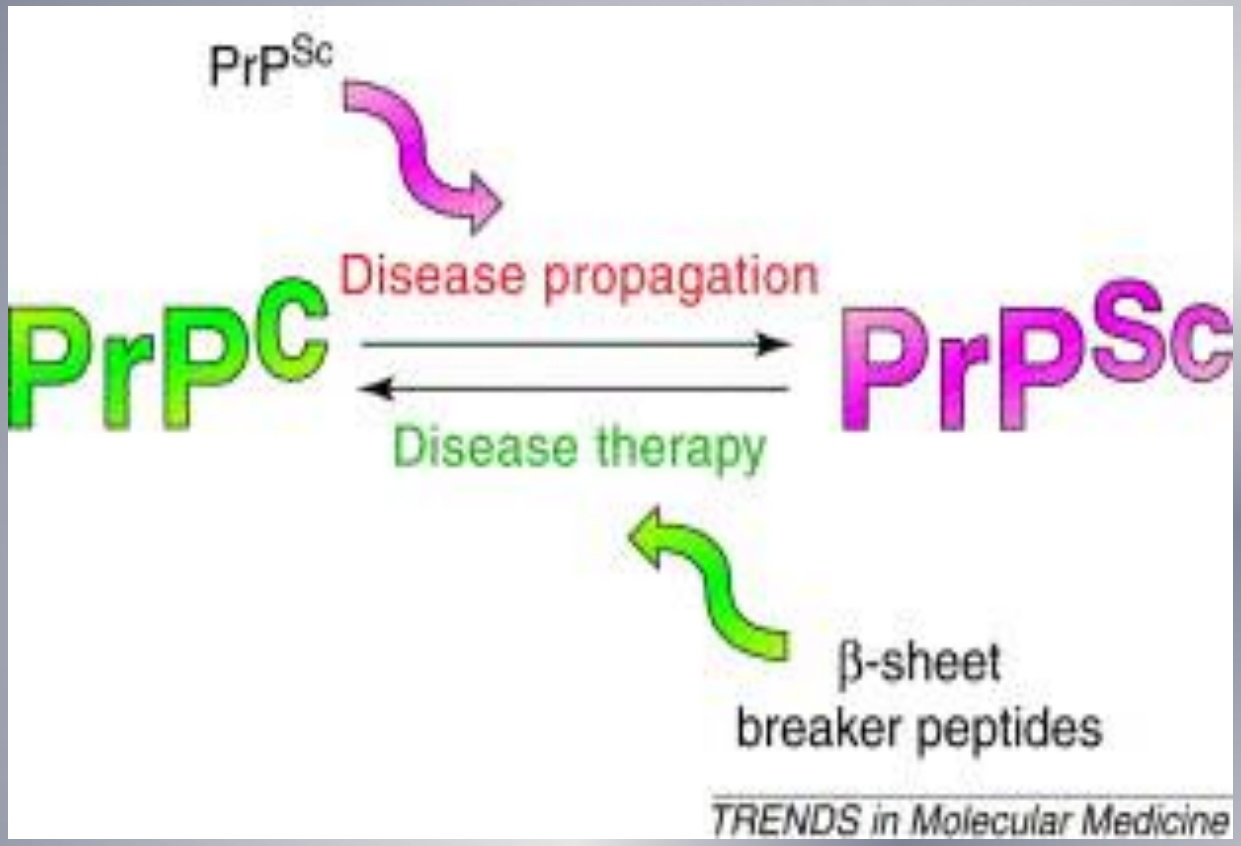
PrP
alpha-helical
protease sensitive

Helical - Happy

PrP^{RES} or PrP^{SC}
beta-pleated sheet
protease resistant

Beta-pleated sheet - Bad

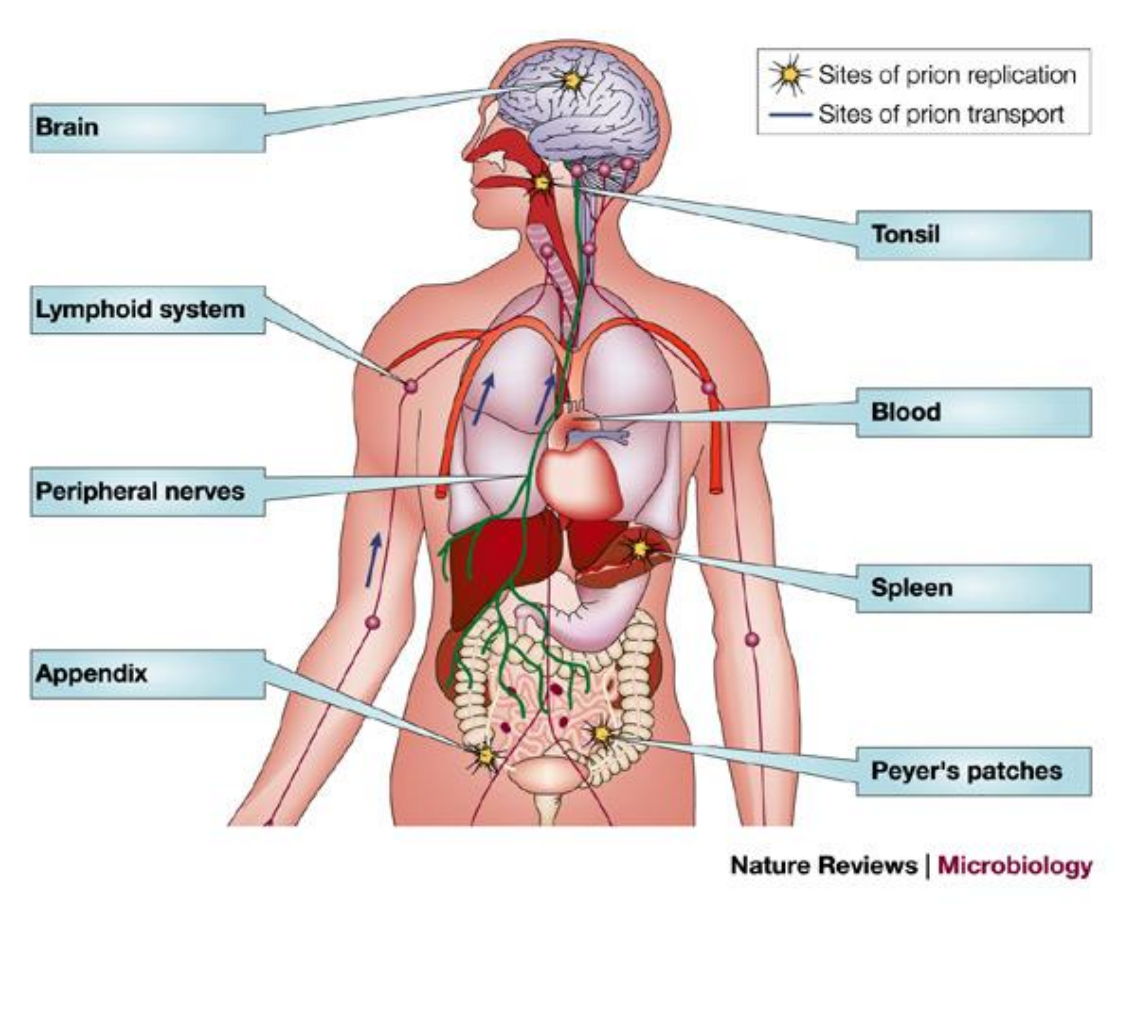

spontaneous conversion
SPORADIC
acquired conversion
ACQUIRED
acquired PrPsc
germline mutation, spontaneous conversion more likely
INHERITED
somatic mutation, spontaneous conversion more likely
SPORADIC


PrPSc
Disease propagation
PrPC
PrPSc
Disease therapy
β-sheet
breaker peptides
TRENDS in Molecular Medicine

Iatrogenic diseases are disorders caused by the treatment of physician or surgeon (iatros from Greek "healer"). The disease develops by transmission of prion-infected material from the (clinically inapparent) donor to the recipient. First case significantly confirmed by animal bioassay was described in a corneal transplant 1974 and another following a neurosurgical procedure in 1977. Over one hundred of cases were detected in recipients of cadaveric, prion infected pituitary hormones (mostly growth hormone or, significantly less, gonadotrophins); the majority in France, UK and USA. Over one hundred of cases were detected following transplantation of prion-infected dura mater (mostly lyophilized commercial preparations) mostly in Japan. Some hormonal as well dura mater cases still occur because of an enormously prolonged incubation period. There are no fresh cases because cadaveric hormones were replaced by synthetic preparations and cadaveric dura mater by autologous tissue (fascia lata, fascia temporalis). Actual problems of iatrogenic prion infections are confined to surgery/neurosurgery, ophthalmology, otorhinolaryngology and dental surgery. Prions have also been detected outside the central nervous system, posterior eye, peripheral nerves, muscles, spleen, lymphoreticular system as tonsils and appendix, intestine, urine (?), olfactory cilia and central olfactory pathway representing a route of infection (nasal secretions). Medical devices in contact with infected tissues became contaminated within minutes. Iatrogenic infections may occur thereafter incubating for years or decades.

Secondary routes of transmission (iatrogenic CJD)

- Transplantes de córnea.
- Paciente operado con material quirúrgico usado en pacientes con CJD.
- Sondas de electroencefalogra-mas utilizadas en pacientes previos.
- Transfusiones de sangre?



Prions are extremely resistant to disinfection and sterilization methods used so far.
They are resistant to proteolytic enzymes and are filtrable.
They survive dry heat at 200 degrees C for 1-2 hours.
Prions are fixed to stainless steel within minutes and remain infectious for long periods. Fixed by desiccation or chemicals may retain infectivity for years.
Their pathogenetic properties depend on tertiary spatial structure (conformation) which is specific and transmissible in experiments.
The prion decontamination appears by far the most important area of the prion science because very little, or nothing, has been done in the majority of world hospitals to prevent iatrogenic transmission.
The number of potentially infectious patients is not known.Therefore, patients undergoing neurosurgery, laryngeal or ophthalmic operations, orthodental treatments and even anaesthetic or endoscopic applications should be classified into risk groups, even if clinically prion disease inapparent.
We consider the high pathogen safety (HPS) autoclave from FEDEGARI as the best actual equipment for the effective decontamination of prions in the hospital practice. The investment costs are moderate and the handling is simple but must be careful. It appears practicable even in small specialized units.

At present there is no routinely available decontamination procedure in washer-disinfectors to allow the reliable inactivation and/or elimination of prions present on reusable surgical instruments. This means that is not possible to provide assurance for preventing iatrogenic transmission of prion diseases. We need effective procedures in prion decontamination that can be integrated into the usual routine of reprocessing surgical instruments. **This article reports on the evaluation of an automated process designed to decontaminate prions** in washer-disinfectors using a quantitative, highly sensitive in vivo assay for surface-adherent 22L prions. The automated process showed great advantages when compared with conventional alkaline cleaning. In contrast, **the new process was as effective as autoclaving at 134 C for 2 h and left no detectable prion infectivity, even for heavily contaminated surfaces.** This indicates a reduction of surface-adherent prion infectivity of >7 log units. Due to its compatibility with even delicate surgical instruments, the process can be integrated into the large scale reprocessing of instruments in a central sterile supply department. The system could potentially make an important contribution to the prevention of iatrogenic transmission of prions.

Prions cause various transmissible spongiform encephalopathies. They are highly resistant to the chemical and physical decontamination and sterilization procedures routinely used in healthcare facilities. The decontamination procedures recommended for the inactivation of prions are often incompatible with the materials used in medical devices. In this study, we evaluated the use of low-temperature hydrogen peroxide gas plasma sterilization systems and other instrument-processing procedures for inactivating human and animal prions. We provide new data concerning the efficacy of hydrogen peroxide against prions from in vitro or in vivo tests, focusing on the following: the efficiency of hydrogen peroxide sterilization and possible interactions with enzymatic or alkaline detergents, differences in the efficiency of this treatment against different prion strains, and the influence of contaminating lipids. We found that gaseous hydrogen peroxide decreased the infectivity of prions and/or the level of the protease-resistant form of the prion protein on different surface materials. However, the efficiency of this treatment depended strongly on the concentration of hydrogen peroxide and the delivery system used in medical devices, because these effects were more pronounced for the new generation of Sterrad technology. The Sterrad NX sterilizer is 100% efficient (0% transmission and no protease-resistant form of the prion protein signal detected on the surface of the material for the mouse-adapted bovine spongiform encephalopathy 6PB1 strain and a variant Creutzfeldt-Jakob disease strain). Thus, gaseous or vaporized hydrogen peroxide efficiently inactivates prions on the surfaces of medical devices.

REVIEW ARTICLE

Prions in dentistry: A need to be concerned and known

B Sushma, Sachin Gugwad[1], Rajdeep Pavaskar[2], Shambhvi A Malik[3]

Departments of Oral and Maxillofacial Pathology, [1]Pedodontics and Preventive Dentistry and [3]Prosthodontics and Crown and Bridge, School of Dental Sciences, Krishna Institute of Medical Sciences Deemed University, Karad, Maharastra, [2]Department of Conservative Dentistry and Endodontics, Goa Dental College and Hospital, Bambolim, Goa, India

ABSTRACT

Prion diseases were first discovered by Stanley B. Prusiner who defined prions as infectious, transmissible proteinaceous particles that lack nucleic acid and are composed exclusively of a modified isoform of the noninfectious cellular prion protein (PrPC). These are incurable neurodegenerative conditions affecting both animals and humans. They may be sporadic, infectious or inherited in origin. Human prion diseases include Creutzfeldt–Jakob desease (CJD), Gerstmann– Straussler–Scheinker disease, Kuru and Fatal familial insomnia. Prions resist the conventional sterilization procedures and hence the dentists must be aware of such diseases so as to opt standard methods of infection control and decontamination for such infectious agents. This review article divulge the dentists with a brief overview of the characteristics of prions, the risk of transmission and the implications for infection control in dentist.

Key words: Prion, prion protein, transmissible spongiform encephalopathies

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journal homepage: www.elsevierhealth.com/journals/jhin

Automated decontamination of surface-adherent prions

A. Schmitt [a,*], I.M. Westner [a], L. Reznicek [a], W. Michels [b], G. Mitteregger [a], H.A. Kretzschmar [a]

SUMMARY

At present there is no routinely available decontamination procedure in washer-disinfectors to allow the reliable inactivation and/or elimination of prions present on reusable surgical instruments. This means that is not possible to provide assurance for preventing iatrogenic transmission of prion diseases. We need effective procedures in prion decontamination that can be integrated into the usual routine of reprocessing surgical instruments. This article reports on the evaluation of an automated process designed to decontaminate prions in washer-disinfectors using a quantitative, highly sensitive *in vivo* assay for surface-adherent 22L prions. The automated process showed great advantages when compared with conventional alkaline cleaning. In contrast, the new process was as effective as autoclaving at 134 °C for 2 h and left no detectable prion infectivity, even for heavily contaminated surfaces. This indicates a reduction of surface-adherent prion infectivity of >7 log units. Due to its compatibility with even delicate surgical instruments, the process can be integrated into the large scale reprocessing of instruments in a central sterile supply department. The system could potentially make an important contribution to the prevention of iatrogenic transmission of prions.

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Photocatalytic degradation of prions using the photo-Fenton reagent

Summary Prions are proteinaceous infectious agents postulated to be the causative agents of a group of fatal neurodegenerative diseases known as transmissible spongiform encephalopathies (TSEs). A known iatrogenic transmission route of TSEs to humans occurs via prion-contaminated surgical instruments or biological materials. Prions, unlike most common pathogens, exhibit an extraordinary resistance to conventional decontami- nation procedures. We have recently demonstrated that the application of TiO2-based heterogeneous photocatalytic oxidation is able to significantly reduce prion infectivity. The present study investigates the potential of a ho- mogeneous photocatalytic method, based on the photo-Fenton reagent, to degrade prion proteins. We show that the photo-Fenton reagent efficiently degrades not only recombinant prion proteins, but also the total protein amount from brain preparations of naturally or experimentally infected spe- cies and PrPSc (PrP scrapie) contained in sheep scrapie brain homogenates.

Enzymatic detergent treatment protocol that reduces protease-resistant prion protein load and infectivity from surgical-steel monofilaments contaminated with a human-derived prion strain

Victoria A. Lawson, James D. Stewart and Colin L. Masters

The unconventional nature of the infectious agent of prion diseases poses a challenge to conventional infection control methodologies. The extraneural tissue distribution of variant and sporadic Creutzfeldt–Jakob disease has increased concern regarding the risk of prion disease transmission via general surgical procedures and highlighted the need for decontamination procedures that can be incorporated into routine processing. In this study, the ability of preparations of enzymatic medical instrument cleaners to reduce the infectivity associated with a rodent-adapted strain of human prion disease, previously reported to be resistant to decontamination, was tested. Efficient degradation of the disease-associated prion protein by enzymatic cleaning preparations required high treatment temperatures (50–60 °C). Standard decontamination methods (1 M NaOH for 1 h or autoclaving at 134 °C for 18 min) reduced infectivity associated with the human-derived prion strain by less than 3 $\log_{10}$ LD_{50}. In contrast, a 30 min treatment with the optimized enzymatic cleaning preparation protocols reduced infectivity by more than 3 $\log_{10}$ LD_{50} and when used in conjunction with autoclave cycles eliminated detectable levels of infectivity. The development of prion decontamination procedures that are compatible with routine cleaning and sterilization of medical and surgical instruments may reduce the risk of the transmission of prion disease in general surgery.

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New hospital disinfection processes for both conventional and prion infectious agents compatible with thermosensitive medical equipment

Summary With the detection of prions in specific tissues in variant and sporadic Creutzfeldt–Jakob diseases, efficient decontamination for human transmissible spongiform encephalopathy (TSE) agents, that is compatible with medical equipment, has become a major issue. We previously described the cleavage of prions on exposure to copper (Cu) and hydrogen peroxide (H_2O_2) and have used this property to develop efficient prion decontamination processes. To validate this approach, in-vitro assays on genuine human and animal prions using both brain homogenates and steel wires to mimic contamination of medical equipment were conducted. In-vivo experiments using steel wire in the hamster 263 K model were then used to evaluate the effect on prion infectivity. Assays on classical pathogens following international norms completed these prion experiments. In-vitro data confirmed the full decontamination efficacy of H_2O_2/Cu on different TSE strains. Combination of Cu with peracetic acid, used for endoscope disinfection, also revealed improved prion decontamination. Animal assay demonstrated efficacy on TSE infectivity of H_2O_2/Cu alone

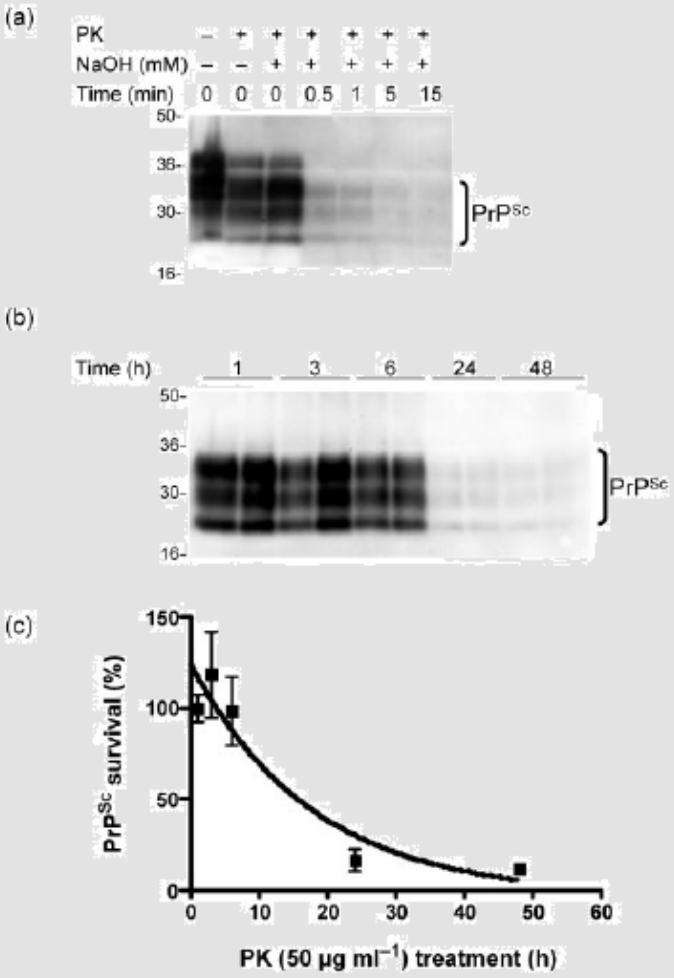

Fig. 1. Western blot analysis of M1000 PrP^{Sc}. M1000 IBH was diluted in distilled water (0.125%, w/v) and incubated in the presence of 25 mM freshly prepared NaOH at room temperature for the times indicated (a). The neutralized solution was treated with PK (100 µg ml^{-1}) and the sample concentrated by centrifugation. Each lane represents 1 mg brain tissue equivalents. Purified M1000 PrP^{Sc} was treated with PK (50 µg ml^{-1}) for the indicated times (b) and quantified relative to a 1 h treatment using a GeneGnome gel documentation system (Syngene) and NIH IMAGE (c). Quantification was based on two independent experiments performed in duplicate. Error bars represent the SEM ($n=4$).

Table 1. Effect of treatment time and temperature on PrP^{Sc} degradation

Results are shown as the amount of M1000 PrP^{Sc} (log_{10}) detected by serial dilution and Western blot analysis of IBH treated with RMEC A or B at low (30 °C) and high (65 °C) treatment temperatures and with increasing treatment times. Total M1000 PrP^{Sc} present in an equivalent IBH after treatment with PK (100 µg ml^{-1}, 30 min, 37 °C) was 3 log_{10}.

Treatment	5 min		30 min	
	30 °C	65 °C	30 °C	65 °C
RMEC A	<3	2.0	2.0	<2
RMEC B	<2.5	1.5	<2.5	<1.5