RABIES

Edited by: Dr.R.G.W.Pinto
Professor & Head
Department of Pathology
Ex Dean Goa University
President Asian Society of Cytopathology

RABIES

Dr.Teresa Noronha Fereira

Rabies is caused by the Lyssavirus sp. Bats and animals like dogs, foxes, racoons, skunks usually are the reservoir for this virus in nature. Humans and other animals like cattle and horses occur as a spill over cross species transmission usually from the bite of dogs and foxes. Bats can transmit the infection through aerosols. Infection in Humans and cattle and horses is usually a dead end infection.

Human rabies is usually fatal. Till date there are reports of survival of only one teenager from this infection. Dog bites are the most common cause of human rabies in our country. The incubation period of the infection is very variable. We have seen cases with incubation period of 3 weeks to upto 22 years. The site of the bite, severity of the bite(degree of bite), vaccination status are the main determinants of the incubation period. Facial and upper limb bites develop rabies faster than lower limb bites.

Clinical presentation:

The clinical presentation can be of two types: 1) furious rabies and 2) dumb rabies

Cases of furious rabies usually present with a prodrome of myalgia with or without low grade fever followed by restlessness, agitation and then symptoms of autonomic nervous system involvement(sweating, tachycardia, hypertension, priapism etc) and finally hydrophobia and aerophobia. These symptoms can evolve over hours to days.

Dumb rabies however has a more gradual onset and progression. The most common differential of dumb rabies is acute inflammatory demyelinating polyneuropathy. Patients present with a gradual lower motor neuron quadriparesis that progresses to bulbar involvement and finally altered sensorium and coma. The red flags that may help to differentiate the two diseases are: 1) onset of weakness in the bitten limb. 2) there are usually paresthesias around the site of the bite prior to the onset of weakness 3) altered sensorium not explained by metabolic reasons.

Diagnosis:

Diagnosis may be made antemortem through nuchal biopsy, analysis of saliva for rabies virus PCR and testing paired serum and csf samples for neutralizing antibodies. Postmortem brain biopsy, partial or complete may be confirmatory in many cases.

Treatment :

Currently no treatment has been shown to be beneficial. Prevention is the only way out.

Prevention:

1) control of animal rabies: this is possible by vaccination of dogs which are the animals at closest quarters to humans. Besides vaccination, limiting roadside dog population is a must as increased stray dog population has been linked to higher incidence of human rabies.

2) Bite area care: All bites should be washed thoroughly under running water for a minimum of 5 minutes with soap, povidone iodine or alcohol solutions. Wound should be left open and not sutured.

3) post exposure prophylaxis: indicated in all category 2 and 3 bites. Post exposure prophylaxis regimen includes vaccination on day 0,3,7,14 and 28. This vaccination may be intramuscular or intradermal. Intradermal injections need to be given in two sites simultaneously but $1/10^{th}$ the volume of intramuscular dose. In Category 3 bites, rabies immunoglobulin is indicated. As much as possible

of the rabies immunoglobulin dose needs to be infiltrated locally around the bite site and the remaining dose injected intramuscular at a site distant to the site of rabies vaccine administration.

4) Preexposure Prophylaxis: this vaccination with rabies vaccine is indicated in people at high risk of contracting the disease due to occupational exposure eg veterinarians and all employees of veterinary clinics. However due to the fatal nature of the disease , there is a suggestion to vaccinate everyone especially I areas with moderate to high risk with high number of stray dogs.

Rabies Scenario in Goa

Rabies is acute encephalitis caused by Rabies lyssavirus of Phylogroup I belonging to Rhabdoviridae family. It is a preventable zoonotic disease that can affect all mammals. Rabies is spread when an infected host transmits the virus by direct contact, by bite wounds and mucosal surface transmission of the virus through its saliva. The pathogenesis and clinical manifestation of Rabies is well documented differentiating it into furious and dumb forms. It is with continuous efforts of vaccination, reporting, testing, preventing spread and education cum awareness that dog mediated Rabies can be prevented.

WHO Technical report Series No. 1012, 2018, stated that India accounted for the most human rabies deaths in Asia (59.9%). Rabies is a notifiable disease of national importance in India. The state of Goa also notified the same through the Act 25 of 1985, Section 57 of the Goa, Daman and Diu Public Health Act 1985.

During the past decade there has been significant progress made by the Department of Animal Husbandry, Government of Goa, in prevention and control of dog mediated Rabies in the State of Goa. This has been possible with several collaborations, deliberations, awareness programs, cooperation from stakeholders and most of all funding from the State and Centre.

The government of Goa along with Mission Rabies (NGO) has been working towards eliminating Rabies in the state of Goa through its joint program, vide MOU (No. 14-9-AH/AH/MR/Part3/2015-16/4123, dt. 05.10.2015) reviewed annually. The focus has been on Rabies elimination from the state of Goa. This has been planned to achieve through:

1. **Mass dog Vaccination (housed & stray dogs)**

Mass dog vaccination was carried out from 2015-2020 throughout the state. Prophylactic immunization was done with Nobivac RABIES (sponsored to Mission Rabies by MSD Animal Health). Vaccination by capture-vaccinate-release methods using manual restrain and nets of owned/pet (house-to-house) and strays dogs was carried out. Teams carried out vaccination and reporting, GPS tagging and details of each dog vaccination by recording offline in a specially designed app and subsequently shared with project managers through an administrator website. Post-vaccination dog-sight surveys were carried out after every vaccination. Vaccination teams rotated through the talukas of Goa yearly, revaccinating dogs over the years.

While Mission Rabies conducted the Mass Dog Vaccination throughout the State, the various Veterinary establishments of the department also continued vaccinating pets. Cattle, were administered only the post bite Rabies vaccination regime as the case arose.

In June 2021, Govt. of Goa declared the state as 'Rabies C*ontrolled*' based on the absence of zoonotic transmission and lack of human deaths due to rabies. Simultaneously, there was also a decrease in the number of positive cases in animals. There was a revised vaccination strategy planned as the central talukas of Goa did not have a single case of dog mediated rabies and sporadic dog mediated rabies cases continue to occur in the northern talukas.

Static Point vaccination was initiated in central talukas, wherein owned and pet dogs were brought on designated days to centers and Veterinary establishments for vaccination. This saved on cost incurred in mobilizing teams for vaccination and has been followed annually for the past three

years. The border talukas continue to be vaccinated by mass dog vaccination method.

2. Enhanced Rabies Surveillance (investigating, testing & reporting)

During the years 2010-15 it was observed that, due to zoonotic nature of the disease, fear of handing and lack of trained manpower for handing and euthanizing rabied animals, identifying to reporting cases, outdated testing method, low vaccination coverage, non adherence to post bite vaccine regime and lack of awareness; the number of animal cases suspected and brain samples tested in the state where only six (6) of which four (4) were positive by Sellar's Staining method, in the state.

The Disease Investigation Unit (DIU), which is a Bio-Safety Level-II laboratory in the state responsible for animal related diagnosis, outbreak investigation, surveillance & reporting, followed the Sellar's stain method prior to December 2016. Identifying "negri bodies" in brain samples for rabies diagnosis was least sensitive in comparison with the OIE (World Organization for Animal Health, formerly the Office International des Epizooties) recommended testing standards.

Mission Rabies (Non-Governmental Organization (NGO), funded by international organizations; Dog Trust & Worldwide Veterinary Service) enhanced its work in the state of Goa in 2016, when it strengthened its team and set up a Rabies Hotline, aiding diagnosis by donating equipment and provided technical/diagnostic support to the state.

Rabies Hotline enhanced reporting of suspected rabid dogs by the public. These cases were attended by the Mission Rabies team. If the animal was

found with signs of Rabies euthanasia and brain sample collected was done followed by carcass disposal. This increased the number of cases reported and brain samples submitted to DIU by Mission Rabies.

The Lateral Flow Test done at the time of post-mortem was confirmed initially by National Institute of Mental health & Neurosciences (NIMHANS), Bengaluru through direct fluorescent antibody testing (dFAT) and PCR. Since December 2016, DIU has been giving confirmed rabies test results by performing dFAT. Annual Proficiency test is also carried out by NIMHANS, Bengaluru.

DIU has been performing dFAT, using Rabies Monoclonal antibody reagent and a fluorescent microscope (Zeiss-PrimoStar), for testing rabies samples. The apple green coloured fluorescence indicates presence of rabies specific antigen in brain tissue by dFAT. The total rabies suspected animal brain samples (dog, cat, cow, jackal, bat, monkey, civet cat) tested at DIU using d-FAT from December 2016 to April 2023 were **892** with a total positive of **184** cases. The decrease in year-wise rabies positivity can be seen at Table 1. Besides testing samples from Goa, DIU has also tested animal brain samples from bordering talukas of Maharashtra and Karnataka (Table.1).

Table. 1. Year-wise Animal Rabies positivity in Goa and animal brain samples tested from bordering talukas of Maharashtra and Karnataka

Year	**2016-17**	**2017-18**	**2018-19**	**2019-20**	**2020-21**	**2021-22**	**2022-23**
Percent Positivity (Goa)	**57.4%**	**58.2%**	**24.6%**	**5.4%**	**14.7%**	**2.5%**	**5.2%**
Total No.of brains tested with positives from Maharashtra	-	1 (1+ve)	2 (2+ve)	3 (3+ve)	1 (1+ve)	2 (2+ve)	59 (36+ve)

& Karnataka							

Human cases of Rabies have drastically decreased from 17 in the year 2014 to nil from 2018 till April 2023. Govt. of Goa has achieved 'Rabies C*ontrolled*' status since June 2021 and has been working towards achieving "*Rabies Free*" status in Goa. Goa rabies surveillance reports indicates a steady decline in canine rabies cases (as shown in Table 1.) but sporadic dog rabies cases continue to occur in the northern talukas (Pernem, Bicholim and Sattari) which have porous borders with neighbouring states of Maharashtra and Karnataka. As the number of positive cases decreases, the control of dog population, vaccination against Rabies, surveillance and awareness efforts continues.

Mission Rabies in partnership with the Government of Goa and Centre for Disease Controland Prevention, USA, identified 104 rabies samples of interest that were collected through canine rabies surveillance, vaccination, and control program in Goa during 2016 to 2018 to perform sequencing. RNA was extracted from dFAT positive samples of dogs, cattle, cats, and jackal at DIU in 2018. Samples were tested by LN34 assay for the presence of rabies virus RNA. Nucleoprotein (N) and glycoprotein (G) gene sequencing was done using the nanopore sequencing protocol (Oxford Nanopore MinION). A total of 80 complete N gene and 97 complete G gene sequences were generated. All Goa sequences generated after sequencing samples clustered within the **Arctic-type 1a rabies virus** variant lineage and were most similar to sequences from the neighboring states of Karnataka, Maharashtra, and Andhra Pradesh (G. Crystal 2020 & Gibson, A.D. et al, 2022). Sequencing of Rabies virus was again done in 2021 and 2022 in partnership with Edinburg University and the results of this are awaited.

3. Mass Education & Awareness in schools, colleges & panchayats

It has been seen that the lack of awareness of the Rabies and its prevention are the major factors leading rabies deaths. Also, children are the major victims of dog bites. The Education & Awareness program was mainly centered on teaching school children about dog behavior, rabies, how to avoid dog bites and what to do if bitten. The program also emphasized the importance and social value of ensuring annual dog vaccination against rabies. Rabies educational messages were spread throughout communities, local authorities and community and public events. All of this was supported and funded by the Department of Animal Husbandry.

Evaluating Status post three years of mass vaccination

In April 2018, Goa State in collaboration with Mission Rabies and Centre for Disease Control and Prevention, USA, conducted a workshop to evaluate status in Stepwise Approach towards dog mediated Rabies Elimination. A day long deliberation was held with Department of Animal Husbandry and the various stakeholders. Goa was at Stage 3, with full scale implementation of the state wide rabies control strategy of the 5 stage process for a country endemic for dog transmitted rabies (Flow Chart attached).

'Rabies C*ontrolled*' status was achieved after several years of vaccination, education and awareness, surveillance and the absence of canine transmitted human deaths due to rabies (2017 till 2022). Goa is now half way through and working towards Stage 4, of the 5 stage process for a country endemic for dog transmitted rabies (Flow Chart attached) wherein the state has to maintain freedom from canine mediated human rabies and elimination of dog rabies.

Surveying, data analysis and the use of modern technology has helped to refine the program and generate quality data. The Dept. of AHVS after revision has signed a MOU with Mission Rabies vide departmental scheme designed to achieve all of the above mentioned points through "*Short Scheme for Mission Rabies"*. This is reviewed annually by District level committee chaired by District Collector & Sate level committee review by Rabies review committee.

Inter-sectoral coordination mechanism (ISC)

Mission Rabies has a hotline number shared with the public in Goa which attends to calls of dogs showing aggression or signs of rabies and responds to it accordingly. There is coordination between Department of A. H. & V. S, Dept. of Health Services and various animal welfare NGO's , Municipalities & Panchayats. The department is in process of formulating the Rabies Action Plan for the State.

With the one health concept in mind the Department has also supported and funded Mission Rabies for Integrated Bite Control Management (IBCM) surveillance. This has helped coordination with the Department of Health Services and achieved an integrated One Health approach.

The Border police at check posts have also been roped in to monitor dog entry at check post. A sign board stating the '*Rabies Controlled*'status of Goa along with Hotline number for reporting and requirement of rabies vaccination of dog entering are installed at border and major tourism destinations in Goa.

Scientific control of stray dog population by Sterilization

Animal birth control programs have been funded by the Department of AHVS to aid local bodies and animal welfare NGOs with technical and financial aid in controlling dog population which is the mandate of the Municipalities & Panchayats.

Controlling rabies in free roaming dogs is expensive and tedious. The state has successfully controlled the disease through vaccination, dog population control, surveillance and education within the state despite the challenges (increase in dog population, labour intensity, and public cooperation) but continues to experience influx of the disease from endemic regions north of Goa.

Challenges faced:

a. High cross border movement of free roaming dogs across Goa–Maharashtra border. Strict control over owner/adopted dog movement in and into state.
b. Low density dog population across large areas with low vaccination coverage due to large free spaces.
c. Higher proportion of stray dogs that do not trust humans, therefore hand catching method is also not effective.
d. Difficulties faced in importing and use of oral bait vaccine to cover dogs that are difficult to catch and hand vaccinate.
e. Awareness and mass vaccination of dogs in other states.
f. Continued mass dog vaccination, surveillance and awareness.
g. Funding for maintenance of controlled status, continued surveillance and improving the laboratory diagnosis.
h. Commitment from all stakeholders involved in maintaining 'Rabies Controlled' status and working towards achieving Rabies Elimination.

Contributed by:
Dr. M. Niceta C. Costa
Veterinary Officer
Disease Investigation Unit
Dept. of Animal Husbanry & Veterinary Services
Tonca, Caranzalem, Goa

Stepwise Approach to Rabies Elimination

Stage 5: Freedom from human and dog rabies

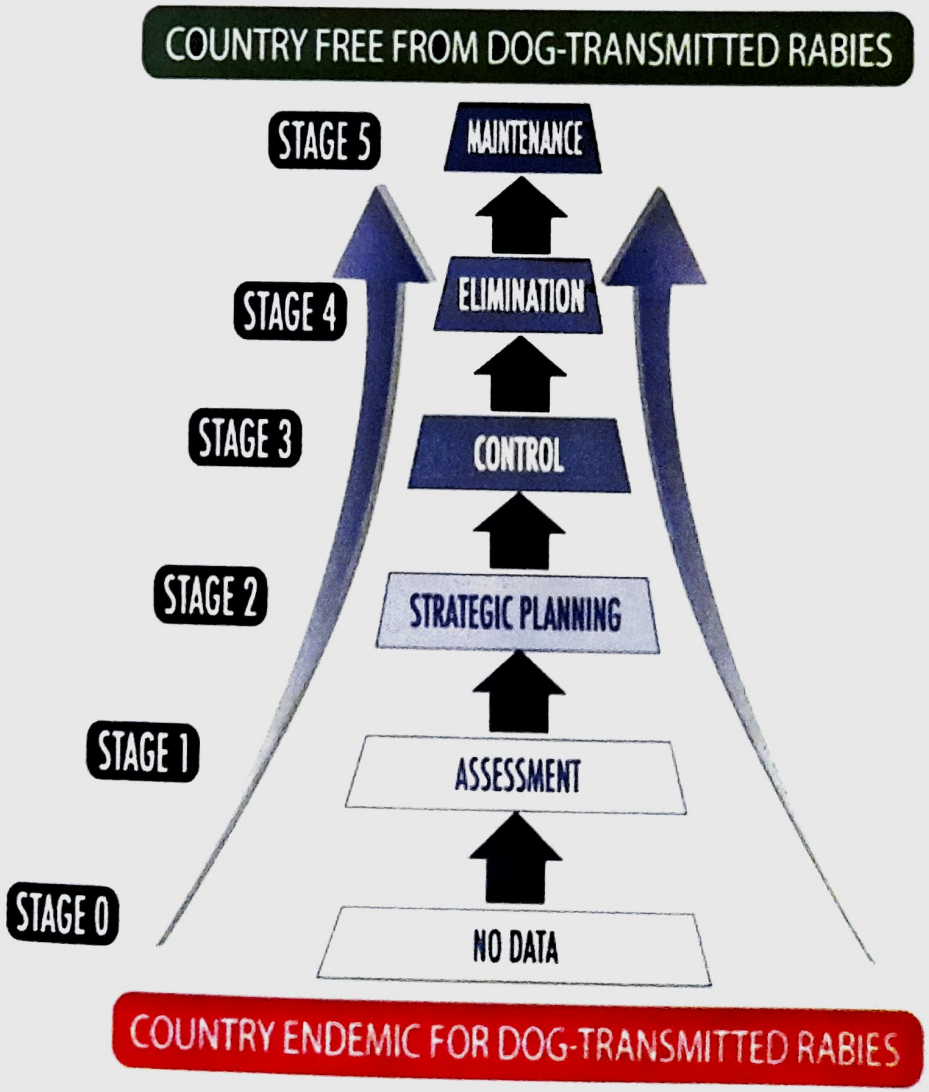
Stage 4: Maintenance of freedom from canine-mediated human rabies; Elimination of dog rabies

Stage 3: Full-scale implementation of the state-wide rabies control strategy

Stage 2: Development of a state-wide rabies control and prevention strategy

Stage 1: Assessment of the local rabies epidemiology, elaboration of a short-term rabies action plan

Stage 0: No information on rabies available, but rabies is suspected to be present

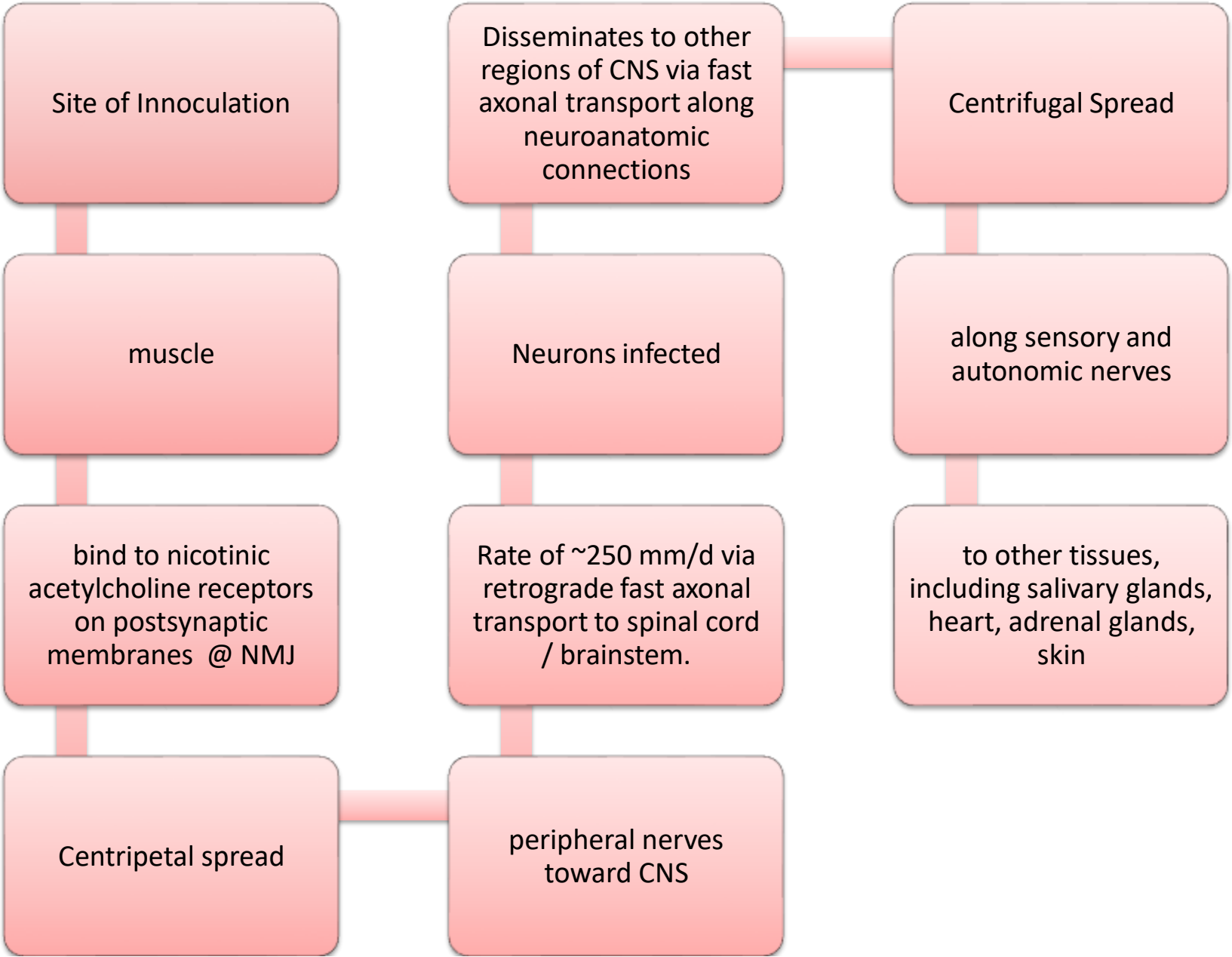


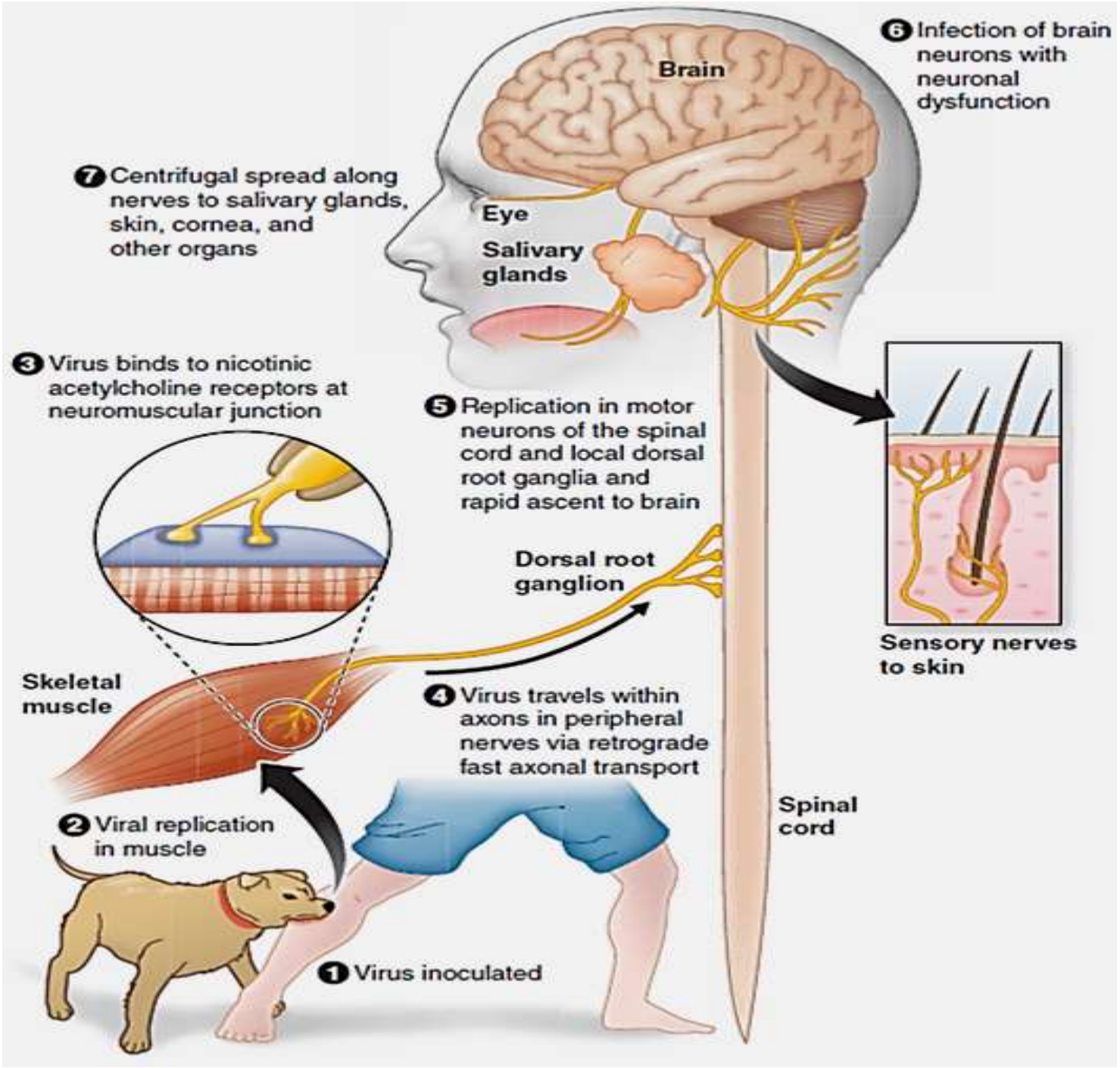
RABIES

DR.G.NAIK

PATHOGENESIS AND CLINICAL FEATURES

PATHOGENESIS:




6 Infection of brain neurons with neuronal dysfunction
Brain
7 Centrifugal spread along nerves to salivary glands, skin, cornea, and other organs
Eye
Salivary glands
3 Virus binds to nicotinic acetylcholine receptors at neuromuscular junction
5 Replication in motor neurons of the spinal cord and local dorsal root ganglia and rapid ascent to brain
Dorsal root ganglion
Sensory nerves to skin
Skeletal muscle
4 Virus travels within axons in peripheral nerves via retrograde fast axonal transport
Spinal cord
2 Viral replication in muscle
1 Virus inoculated

Microscopy:

- mild inflammatory changes in the CNS in with mononuclear inflammatory infiltration in the leptomeninges
- perivascular regions, and parenchyma, including microglial nodules called Babes nodules
- most characteristic pathologic finding in rabies is Negri body
- Negri bodies are not observed in all cases of rabies.

- Negri bodies are eosinophilic cytoplasmic inclusions in brain neurons that are composed of rabies virus proteins & viral RNA

- Inclusions = minority of infected neurons & commonly observed in Purkinje cells of cerebellum and in pyramidal neurons of the hippocampus, and are less frequently seen in cortical and brainstem neurons

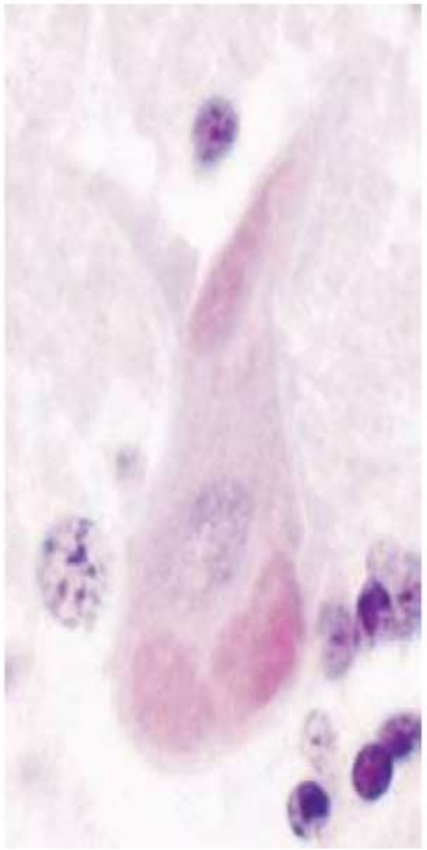
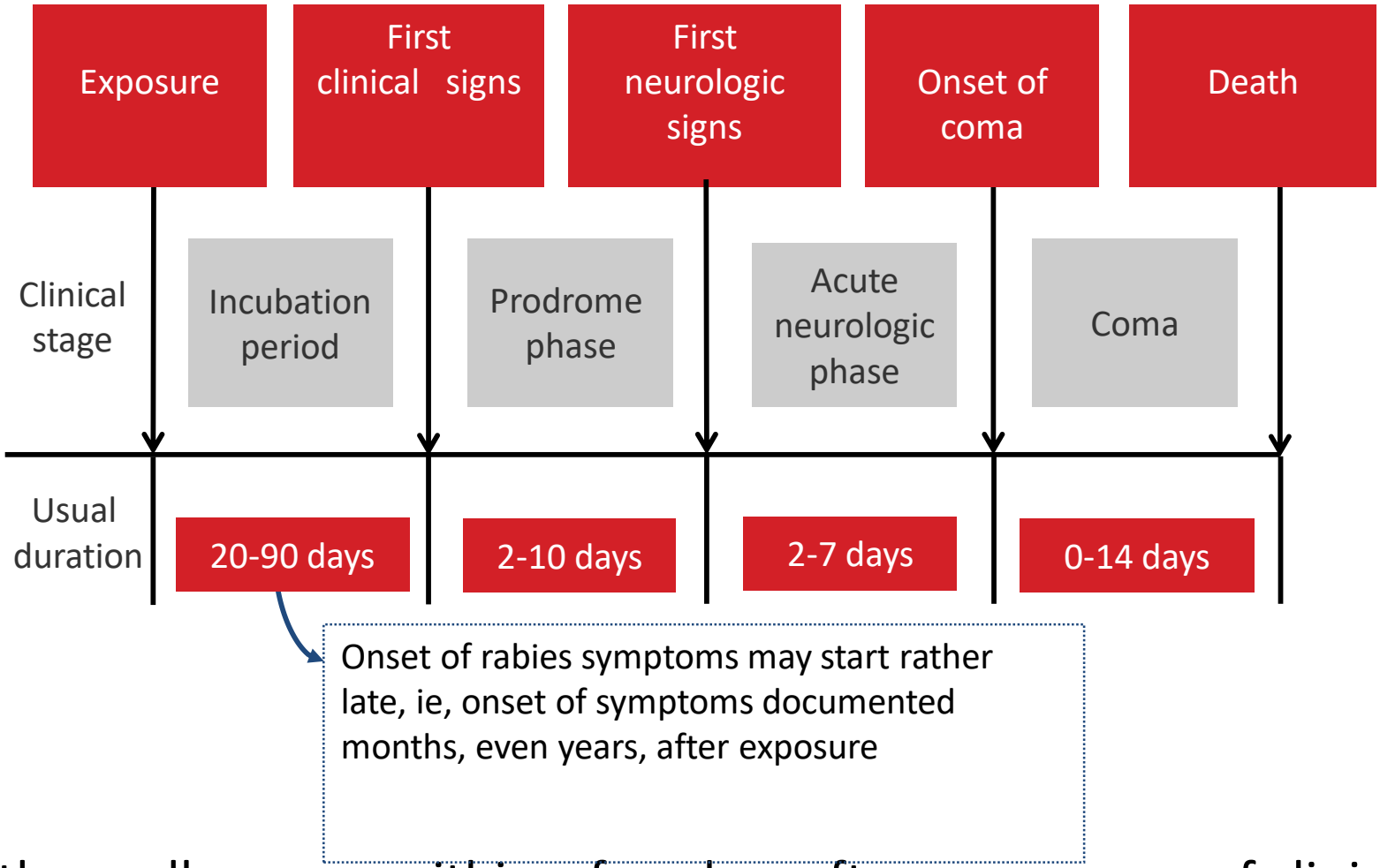


FIGURE 232-3 Three large Negri bodies in the cytoplasm of a cerebellar Purkinje cell from an 8-year-old boy who died of rabies after being bitten by a rabid dog in Mexico. *(From AC Jackson, E Lopez-Corella: N Engl J Med 335:568, 1996. © Massachusetts Medical Society.)*

C/F:

- usually presents ➡ atypical encephalitis with relative preservation of consciousness
- may be difficult to recognize late in the clinical course when progression to coma has occurred
- Minority of patients present with acute flaccid paralysis.
- There are 1. Prodromal
  2. Acute neurologic
  3. Comatose phases that usually progress to death despite aggressive therapy.

Human rabies: Clinical stages



Death usually occurs within a few days after appearance of clinical s/s

PRODROME:

- Nonspecific
- Including fever, malaise, headache, nausea, and vomiting
- Anxiety or agitation may also occur.
- Earliest specific neurologic symptoms → paresthesias, pain or pruritus near site of exposure, one or more of which occur in 50–80% of patients and strongly suggest rabies.
- The wound → usually healed by this point
- These symptoms → reflect infection with asso. Inflamm. changes in local dorsal root / Cranial sensory ganglia.

Encephalitic Rabies:

Manifestations include:

- Fever, confusion, hallucinations, combativeness, seizures
- Autonomic dysfunction common → hypersalivation, gooseflesh, cardiac arrhythmia, priapism.
- Episodes of hyperexcitability typically followed by periods of complete lucidity that become shorter as disease progresses
- Early brainstem involvement → classic hydrophobia (involuntary, painful contraction of diaphragm & accessory respiratory, laryngeal, and pharyngeal muscles in response to swallowing liquids)

Contd:

- Aerophobia (the same features caused by stimulation from a draft of air)
- D/t dysfunction of infected brainstem neurons that normally inhibit inspiratory neurons near the nucleus ambiguus, resulting in exaggerated defense reflexes that protect respiratory tract
- Combination of hypersalivation & pharyngeal dysfunction responsible for classic appearance of "foaming at the mouth"
- Brainstem dysfunction progresses rapidly, & coma—followed within days by death.

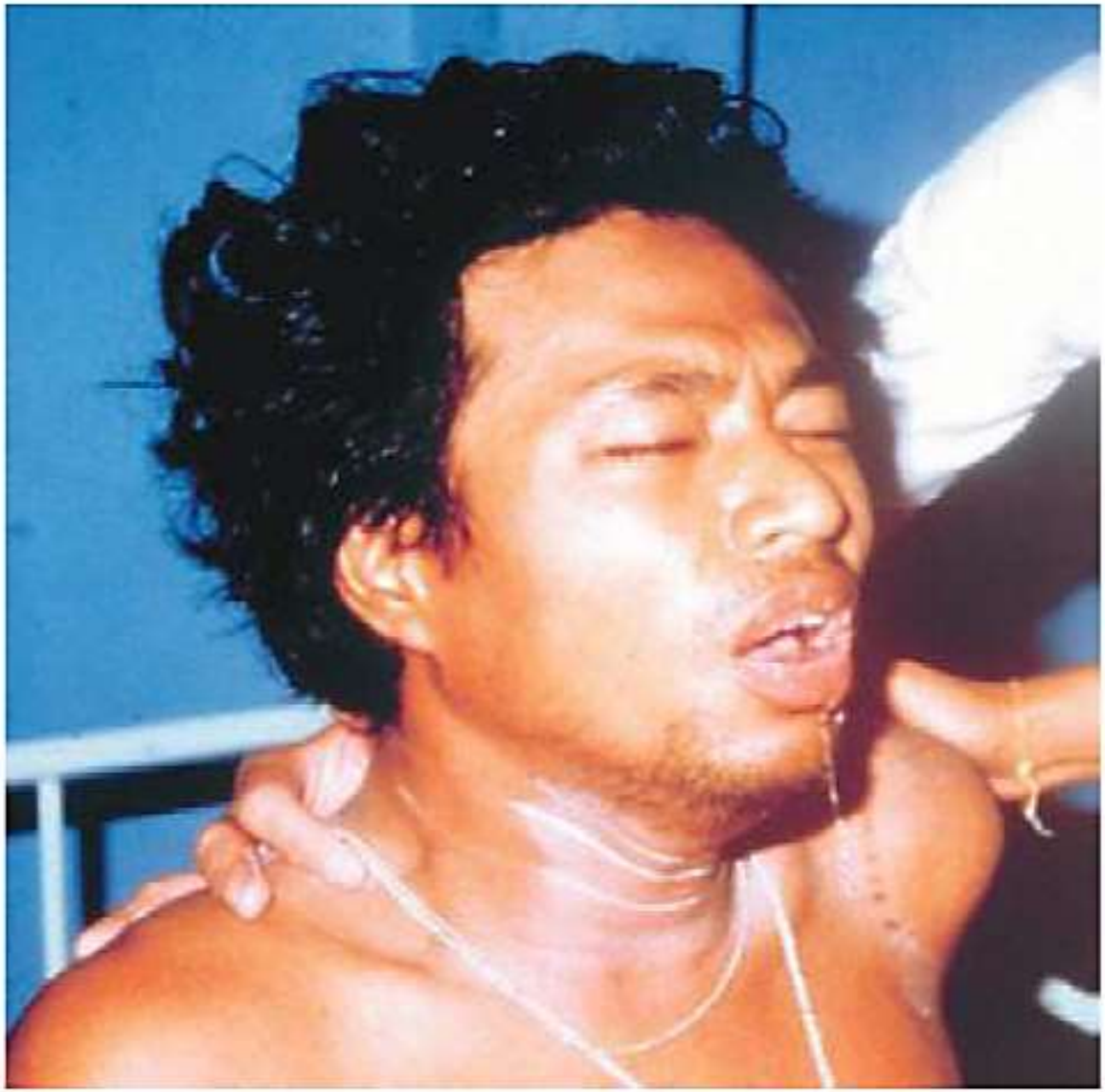


FIGURE 232-4 Hydrophobic spasm of inspiratory muscles associated with terror in a patient with encephalitic (furious) rabies who is attempting to swallow water. *(Copyright DA Warrell, Oxford, UK; with*

Late complications include:

- Cardiac and/or respiratory failure, disturbances of water balance (SIADH or diabetes insipidus)
- Noncardiogenic pulmonary edema
- Gastrointestinal hemorrhage.
- Cardiac arrhythmias may be d/t dysfunction affecting vital centers in the brainstem or to myocarditis.
- Multiple-organ failure

Paralytic Rabies:

- Muscle weakness predominates & cardinal features of encephalitic rabies (hyperexcitability, hydrophobia, and aerophobia) are lacking.
- Early & prominent flaccid muscle weakness, often beginning in the bitten extremity and spreading to produce quadriparesis and facial weakness.
- Sphincter involvement common
- Sensory involvement usually mild
- Cases are commonly misdiagnosed as GB syndrome.
- Survive a few days longer than those with encephalitic rabies, but multiple-organ failure nevertheless ensues.

TABLE 232-1 CLINICAL STAGES OF RABIES

Phase	Typical Duration	Symptoms and Signs
Incubation period	20–90 days	None
Prodrome	2–10 days	Fever, malaise, anorexia, nausea, vomiting; paresthesias, pain, or pruritus at the wound site
Acute neurologic disease		
Encephalitic (80%)	2–7 days	Anxiety, agitation, hyperactivity, bizarre behavior, hallucinations, autonomic dysfunction, hydrophobia
Paralytic (20%)	2–10 days	Flaccid paralysis in limb(s) progressing to quadriparesis with facial paralysis
Coma, death[a]	0–14 days	

[a]Recovery is rare.

Source: MAW Hattwick: Rabies virus, in *Principles and Practice of Infectious Diseases,* GL Mandell et al (eds). New York, Wiley, 1979, pp 1217–1228. Adapted with permission from Elsevier.

Rabies in children

- Due to their short stature, children are susceptible to bites on face, scalp, and upper part of the body.
- Children are more susceptible to animal bites by playing in open grounds or in streets.
- Children cannot ward off animals easily.
- Children are more likely to provoke animals.
- Children might not report a bite or scratch.
- 40-60% of all animal bite cases are reported to occur in children <15 years of age.

RABIES VIRUS STRUCTURE

DR GREESHMA THAMPAN

2ND YEAR JUNIOR RESIDENT

DEPARTMENT OF MICROBIOLOGY

FAMILY : RHABDOVIRIDAE

GENUS : LYSSAVIRUS

(Bullet shaped 45 nm - 100 nm in diameter)

ENVELOPED

HELICAL NUCLEOCAPSIDS

SINGLE STRANDED NEGATIVE SENSE RNA

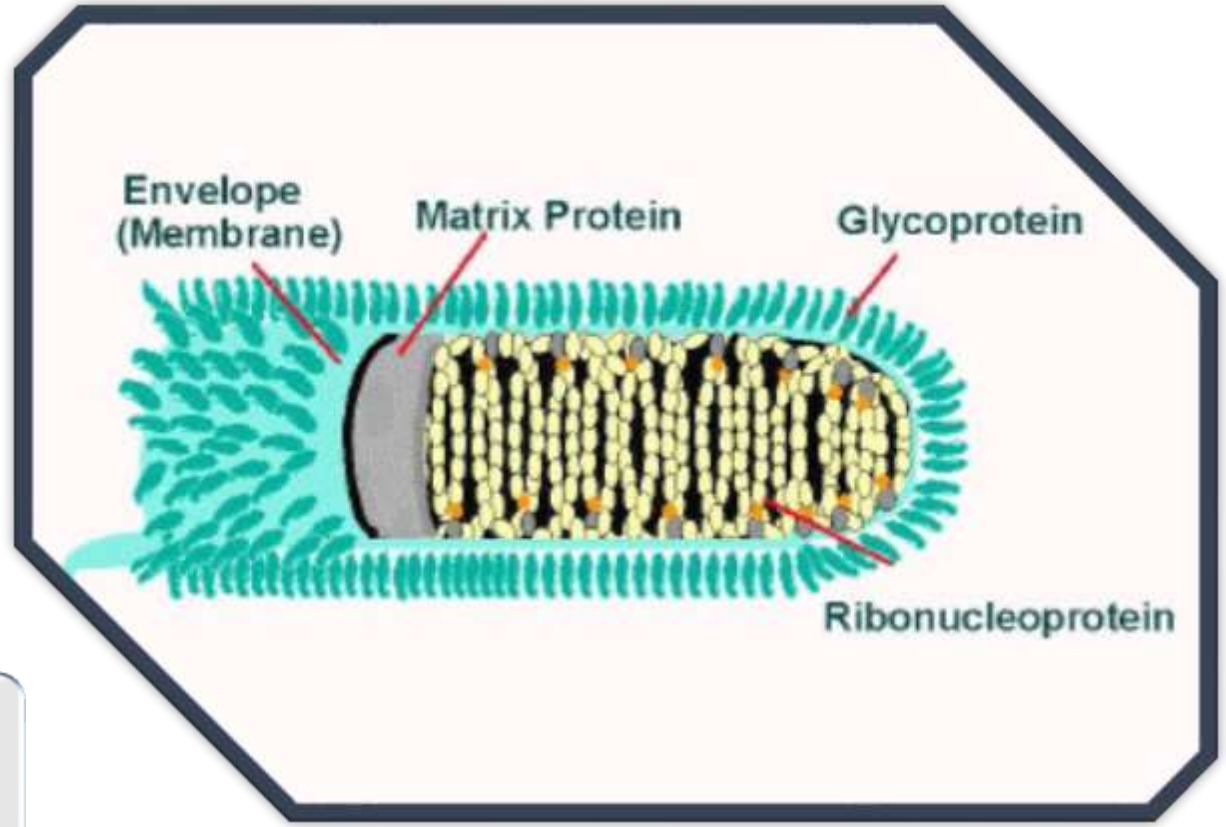
ENCODE

- 3 structural proteins
- 2 non structural proteins

ENVELOPE

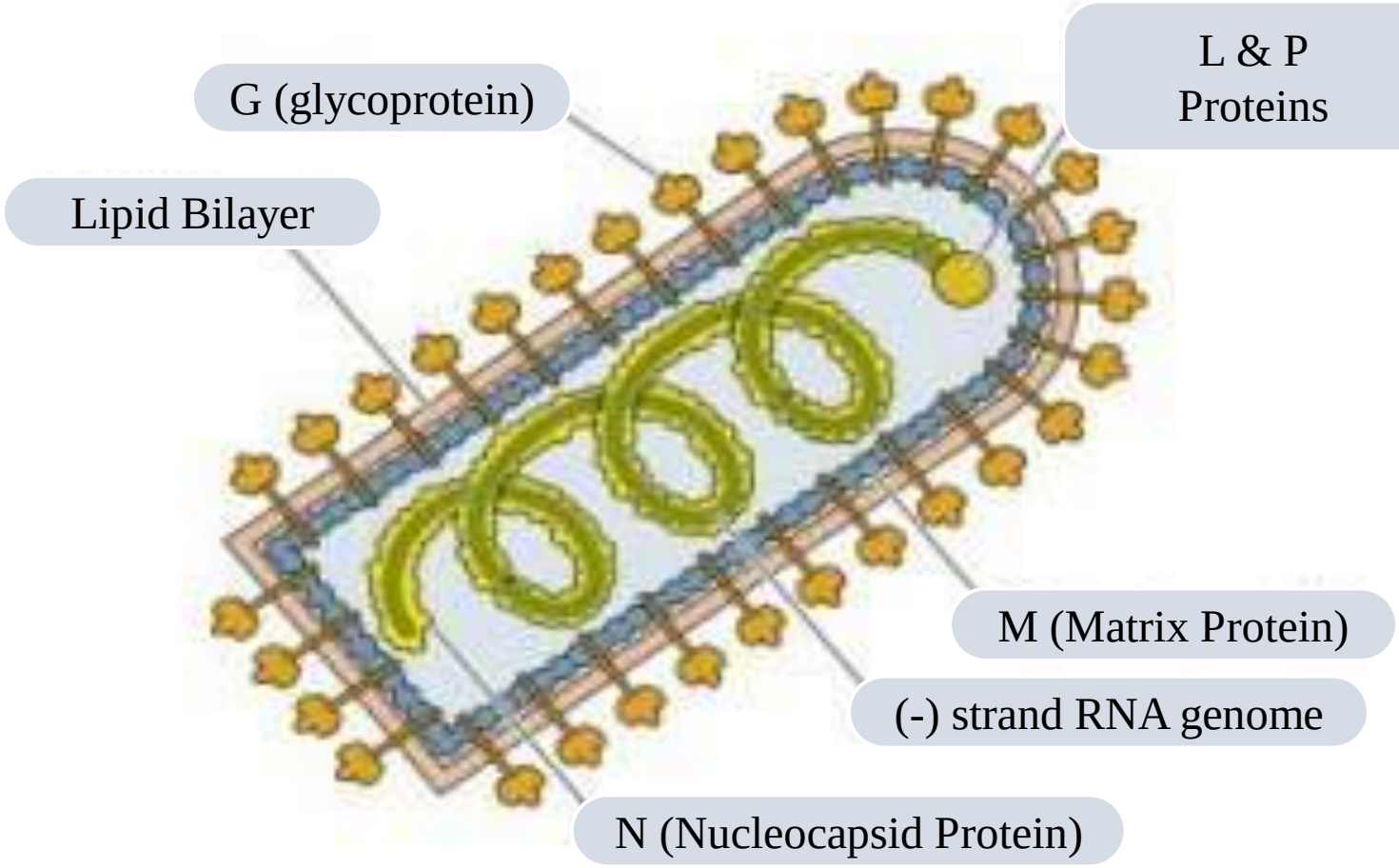
BILIPID LAYER

- M protein (Matrix protein)
- G protein (Glycoprotein)
  - Bind to the receptor of the host cell

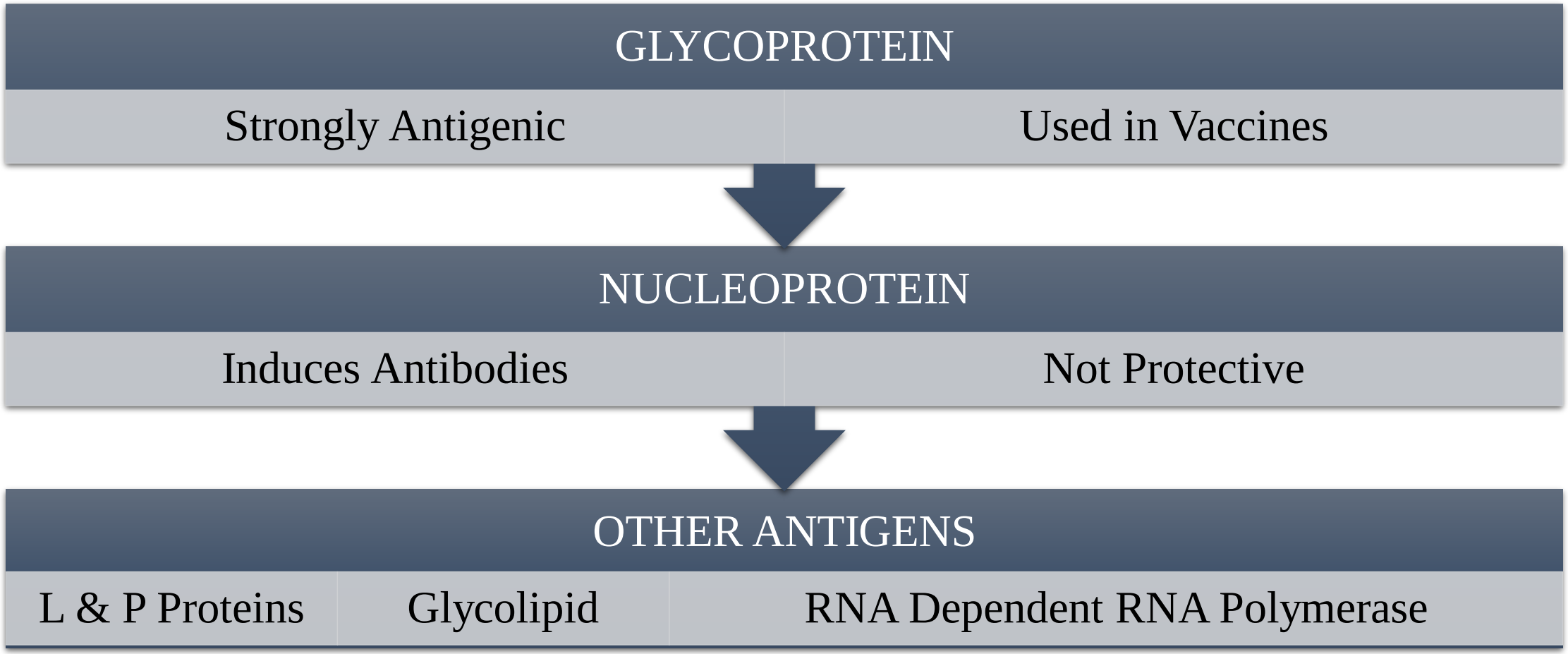


NUCLEOCAPSID PROTEIN

Protect RNA from hydrolysis



ANTIGENIC PROPERTIES



STRUCTURAL PROTEINS

G protein

- Glycoprotein – binds to nicotine ACH receptors on host cell

M protein

- Matrix Protein

N protein

- Nucleocapsid Protein

GLYCOPROTEIN

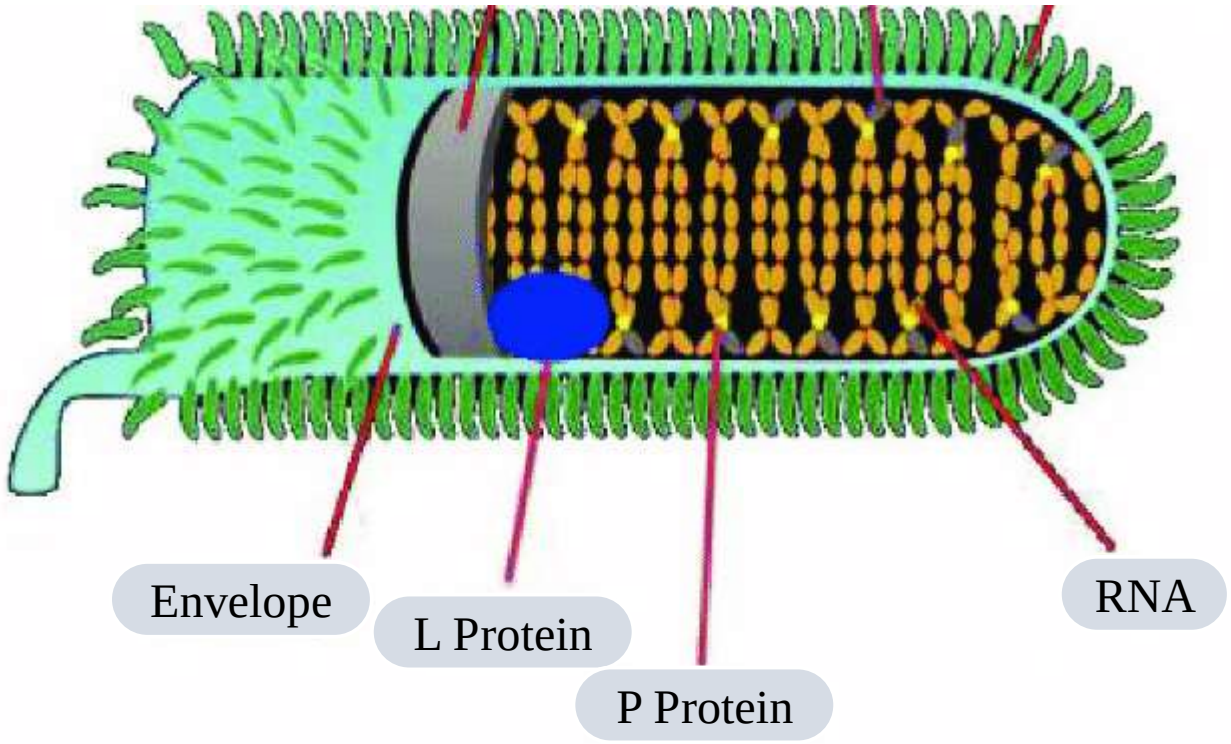
- Surface spikes
- Pathogenic
- Mediates binding of virus to acetylcholine receptors in neural tissue
- Diagnostic role
  - Antibody detection by hemagglutination inhibition test
- Induces neutralizing antibodies
- Stimulates cytotoxic t cells
- Purified glycoprotein may act as safe, effective subunit vaccine

NUCLEOPROTEIN

- It is a nucleocapsid
- Induces complement fixing antibodies
- Antibodies are non protective
- Antigen is group specific & cross reactions do occurs with rabies related virus
- Antiserum prepared against the nucleocapsid antigen is used in diagnostic immunofluorescence tests

NON STRUCTURAL PROTEIN

- L protein
- P protein



TYPES OF RABIES VIRUS

Street virus

- Recovered from naturally occurring cases of rabies
- Affects salivary glands
- Pathogenic, produce intracytoplasmic inclusion bodies
- Incubation period (1 to 3 months)

Fixed virus

- It has a short, fixed and reproducible incubation period
- Prepared by repeated culture in brain of rabbit
- Pathogenic for Humans under certain conditions
- Incubation period (4 to 6 days)
- Used for preparation of anti-rabies vaccine

RABIES VACCINES AND IMMUNOGLOBULINS

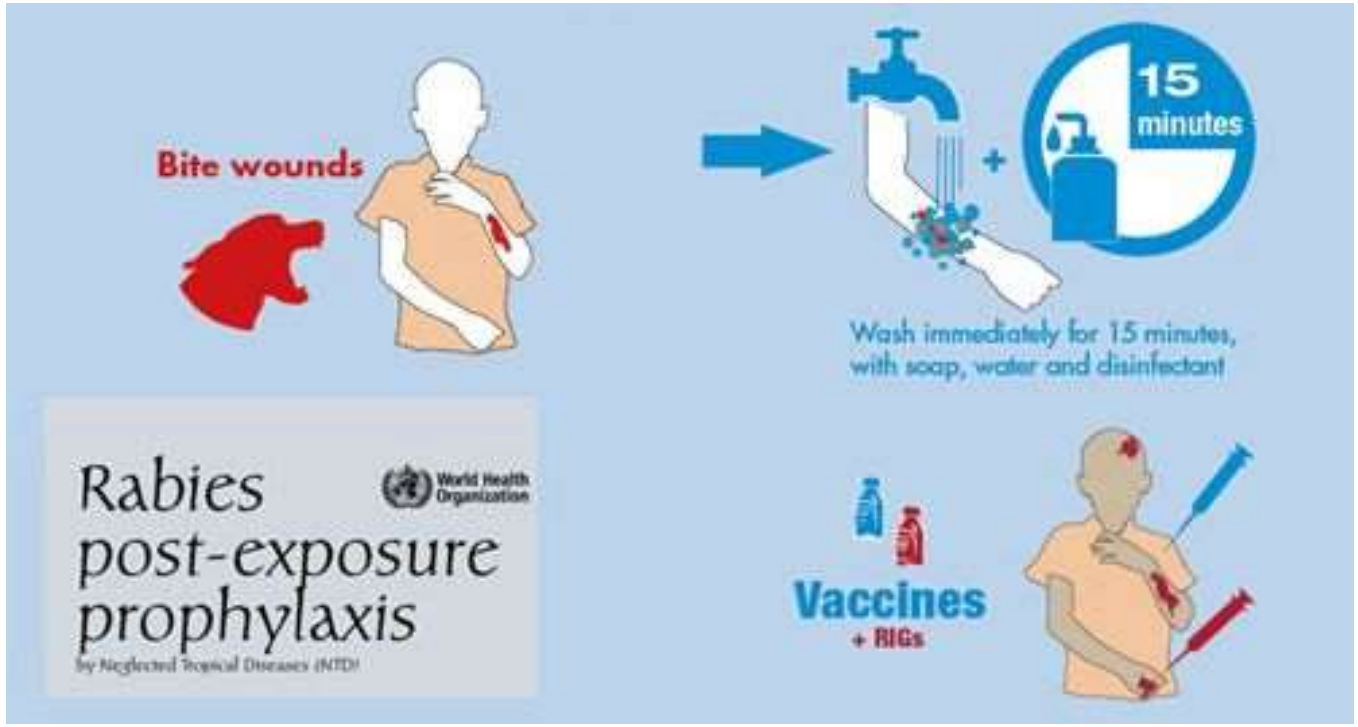
DR. VISHRUTI P RAIKAR

DR. IAN PEREIRA, Associate professor

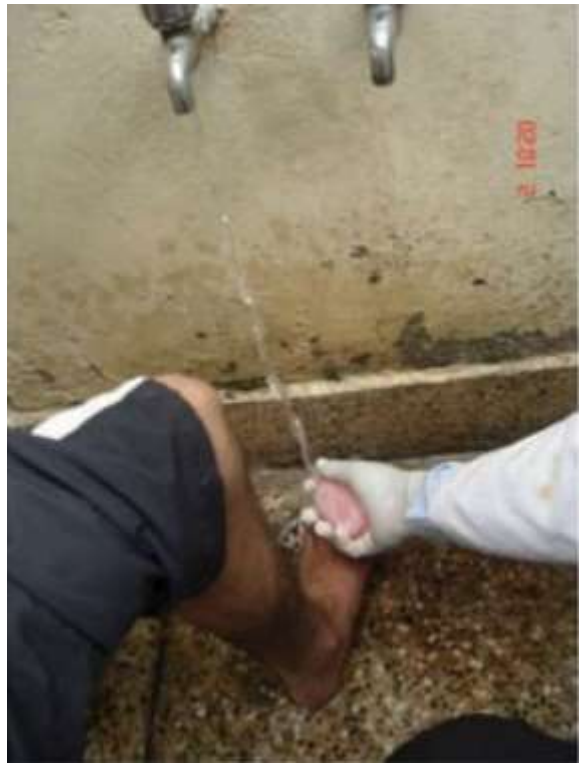
DEPARTMENT OF PHARMACOLOGY

POST EXPOSURE PROPHYLAXIS

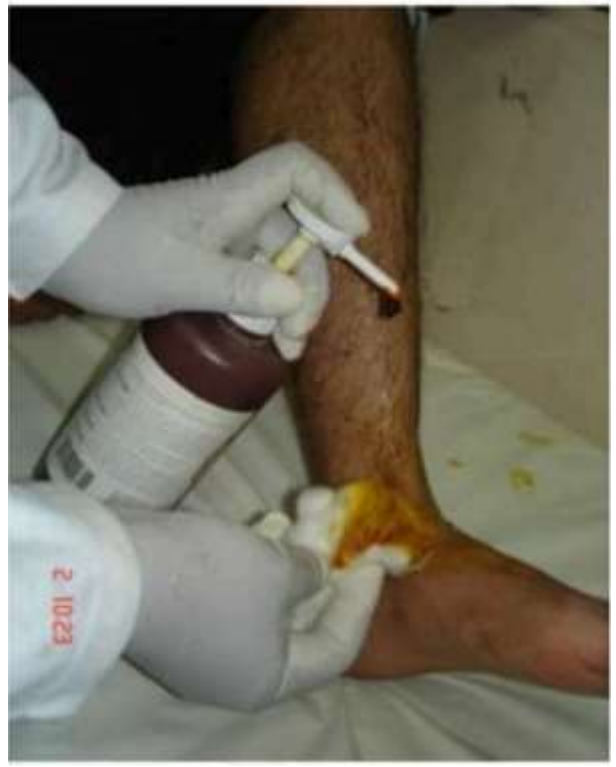
- Management of animal bite wound (s)
- Passive immunization with rabies immunoglobulin (RIG)
- Active immunization with anti – rabies vaccine (ARV)



Management of animal bite wound (s)

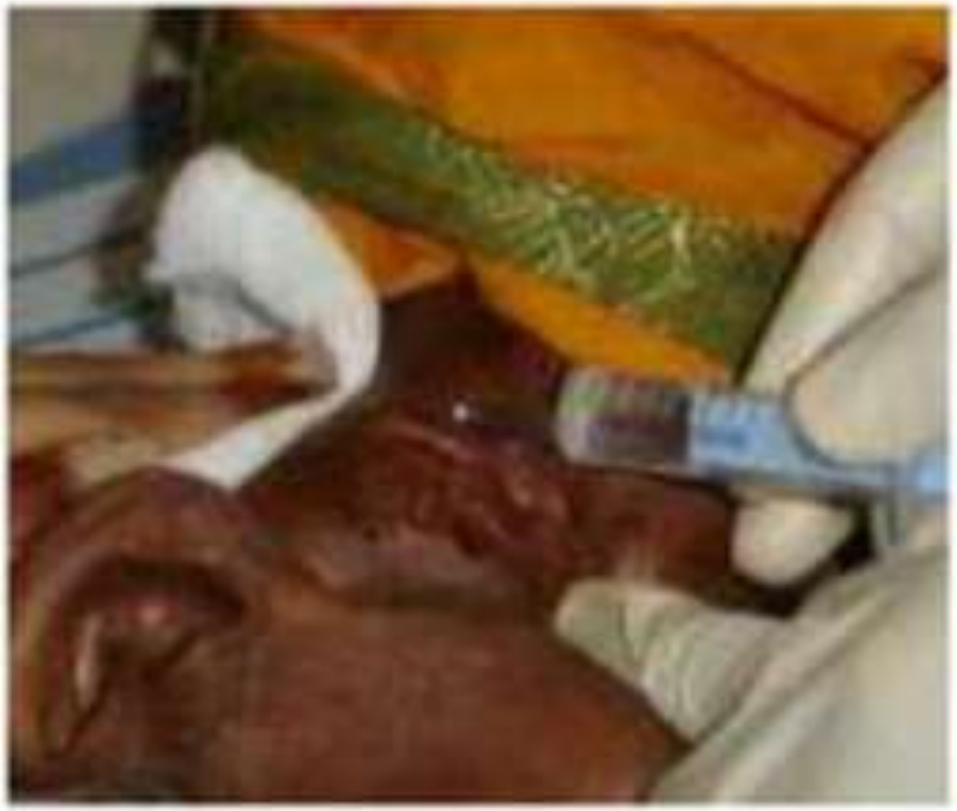


Mechanical removal of virus from the wound (s)



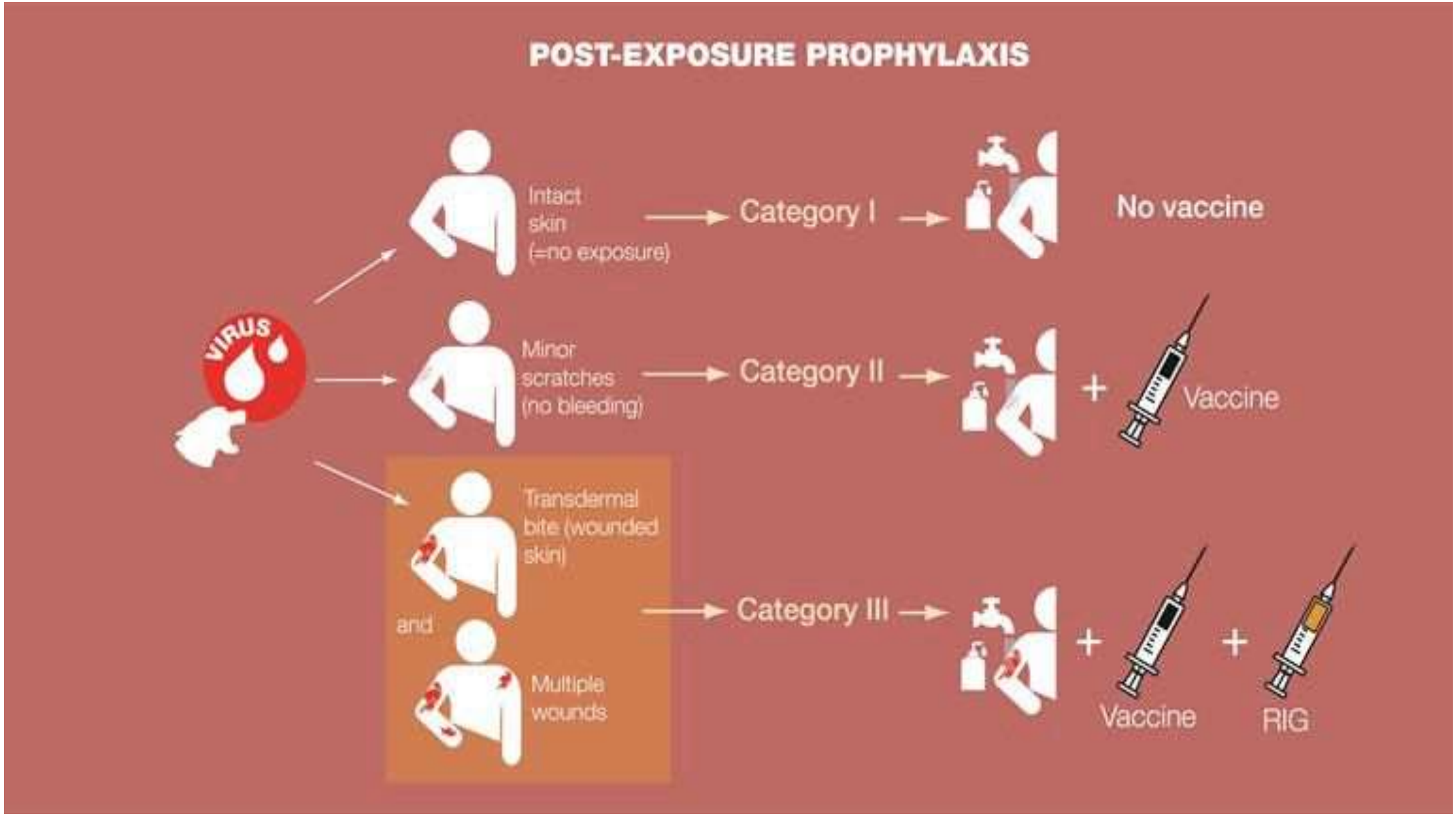
Inactivation of the virus

Neutralization of the virus



- application of antiseptics
- Local infiltration of rabies immunoglobulin
- Tetanus prophylaxis

DO NOT SUTURE OR COVER THE WOUND


POST-EXPOSURE PROPHYLAXIS
VIRUS
Intact skin (=no exposure)
Category I
No vaccine
Minor scratches (no bleeding)
Category II
+
Vaccine
Transdermal bite (wounded skin)
and
Multiple wounds
Category III
+
Vaccine
+
RIG

Category of bites (WHO)

Category I	• Touching or feeding of animals • Licks on intact skin • Contact of intact skin with secretions/ excretions of rabid animal/human case
Category II	• Nibbling of uncovered skin • Minor scratches or abrasions without bleeding
Category III	• Licks on mucous membrane • Single or multipe transdermal bites or scratches, licks on broken skin

Recommended treatment

Category I	• None
Category II	• Local Rx of wounds • Anti rabies vaccine
Category III	• Local Rx of wounds • Anti rabies vaccine • Rabies immunoglobulin

Rabies immunoglobulin (RIG)

- Ready – made anti –rabies antibodies , to tide over the initial phase of infection
- Binds with the rabies virus resulting in neutralization and thus loss of infectivity
- Infiltrated locally at the site of wound / bite
- Two types available:
- A) equine rabies immunoglobulin (ERIG)
- B) human rabies immunoglobulin (HRIG)
- Indicated in 1) all category 3 exposures
- 2) both category 2,3 exposures in immunocompromised patients.

EQUINE RABIES IMMUNOGLOBULIN

- Heterologous ,produced by hyperimmunization of equines.
- Highly purified Fab 2 fragments
- Small risk of anaphylactic reaction
- Dose of ERIG is 40 IU per kg body weight of patient
- Preparations contain 300 IU of immunoglobulin per ml

Currently available ERIG in India

Brand	Product	Pharmaceutical
Anti-Rabies Serum (ARS)	Purified equine RIGs, 5 ml vial (300 IU/ml, 1500 IU potency)	Central Research Institute, Kasauli, Himachal Pradesh
Equirab	Purified Equine RIGs, 5ml vial (300 IU/ml, 1500 IU potency)	Bharat Serums and Vaccines Limited, Mumbai
Vinrig	Purified Equine RIGs, 5ml vial (300 IU/ml, 1500 IU potency)	VINS Biopharma, Hyderabad
Abhayrig	Purified Equine RIGs, 5 ml vial (300 IU/ml, 1500 IU potency)	Human Biologicals Institute, Hyderabad

Human Rabies Immunoglobulin

- Homologous origin , relatively free from the side effects
- Expensive and is imported from other countries
- Longer half life given at half the dose of equine anti- rabies serum
- Dose of HRIG is 20 IU per kg body weight
- HRIG preparation is available in concentration of 150 IU per ml

Currently Available HRIG In India

Brand	Product	Pharmaceutical
Berirab-P	Human Rabies Immunoglobulin, 150IU/ml; 2 ml (300 IU) ampoule & 5 ml (750 IU) ampoule	ZLB Behring AG,Marburg, Germany/Bharat Serums and Vaccines Ltd., Mumbai.
Imogamrab	Human Rabies Immunoglobulin, 150IU/ml; 2 ml (300 IU) ampoule & 5 ml (750 IU) ampoule	Sanofi Pasteur, France
Kamrab	Human Rabies Immunoglobulin, 150 IU/ml; 2 ml (300 IU) vial and 5 ml (750 IU) vial	Kamada Ltd.,Beit-Kama, Israel /Synergy Diagnostics Pvt. Ltd.,Thane,Maharashtra

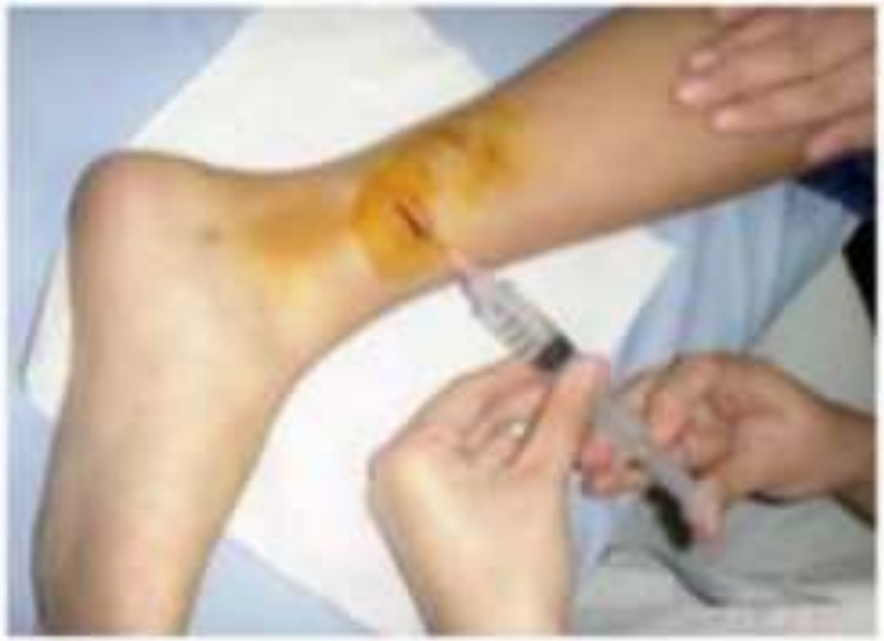
Administration Of RIG

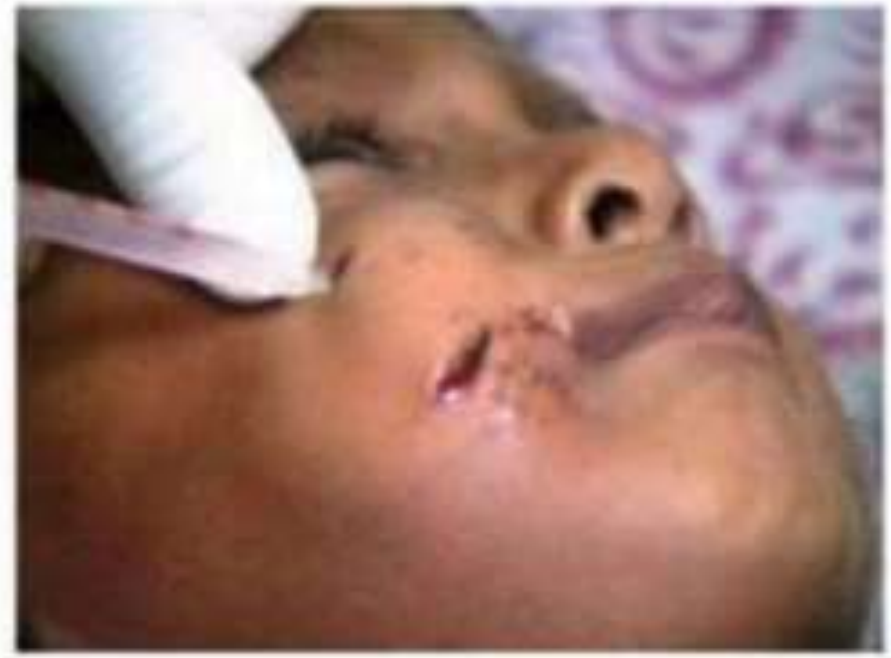
Do's

- Should be brought to room temperature before administration
- As much of the dose is anatomically feasible should be infiltrated into and around the wound
- Remaining dose to be administered by deep IM at a site distant from the vaccine injection site
- If multiple wounds present , calculated volume to be diluted in sterile normal saline to a volume sufficient to infiltrate all wounds
- Can be administered up to the seventh day after the first dose of ARV
- Tip of finger and toe , ear lobe or bites on nose or around the eye should be carefully infiltrated without excessive pressure
- Patient kept under observation for atleast half an hour after administration of ERIG

DON’T’S

- Multiple needle injections into the wound should be avoided.
- Total recommended dose of RIG must not be exceeded , because it will suppress the antibody production
- Should never be administered in the same syringe or at the same anatomical site as the vaccine





Anti Rabies Vaccines

- Nerve tissue vaccines used previously
- Reactogenic and less immunogenic
- Production was stopped in December , 2004
- Now , cell culture vaccine (CCVs) and purified duck embryo vaccines (PDEV) are now used for active immunization
- Given as one single intramuscular dose with potency of > 2.5 IU per IM dose
- CCVs approved for intradermal could be given ID
- Adverse events following immunization (AEFI) very minimal with CCVs & PDEVs

VACCINES

1. cell culture vaccines

- Human Diploid Cell Vaccine (HDCV), Liquid (Adsorbed),

1ml: Produced locally in private sector

- Purified Chick Embryo Cell Vaccine (PCECV), 1ml: Produced locally in private sector
- Purified Vero Cell Rabies Vaccine (PVRV), 0.5ml and 1ml: Imported and also produced locally in public & private sectors

2. Purified Duck Embryo Vaccine (PDEV), 1ml:

- Produced locally in private sector

Currently available anti-rabies vaccines in India

Brand	Product	Pharmaceutical
Abhayrab*	Purified Vero cell Rabies Vaccines (PVRV)	Human Biologicals Institute, Hyderabad
Indirab*	Chromatographically purified (PVRV)	Bharat Biotech International Ltd, Hyderabad
PVRV	Purified Vero cell Rabies Vaccine (PVRV)	Pasteur Institute of India, Coonoor, Tamilnadu
Rabipur*	Purified Chick Embryo Cell Vaccine (PCECV)	Novartis Vaccines, Mumbai
Rabivax	Human Diploid Cell Culture Vaccine (HDCV) (Liquid)	Serum Institute of India, Pune
Vaxirab	Purified Duck Embryo Vaccine (PDEV)	Zydus Health Care ltd., Ahmedabad
Vaxirab-N*	Purified Chick Embryo Cell Vaccine (PCECV)	Zydus Health Care Ltd, Ahmedabad
Verorab*	Purified Vero cell Rabies Vaccines (PVRV)	Sanofi Pasteur/ Zuventus Health Care, Mumbai

Storage, Transport & Reconstitution

- Most CCVs and PDEV are stored and marketed in freeze dried (lyophilized) form
- But vaccines should be kept and transported at a temperature range of 2-8ºC and protected from sunlight
- Reconstituted with the diluent prior to use
- IM dose to be given immediately after reconstitution
- Should not be used after 8 hours of reconstitution
- For ID administration, vaccine vial should be stored at 2-8ºC after reconstitution and total content should be used at the most within 8 hours

Immunity & Antibody Titre

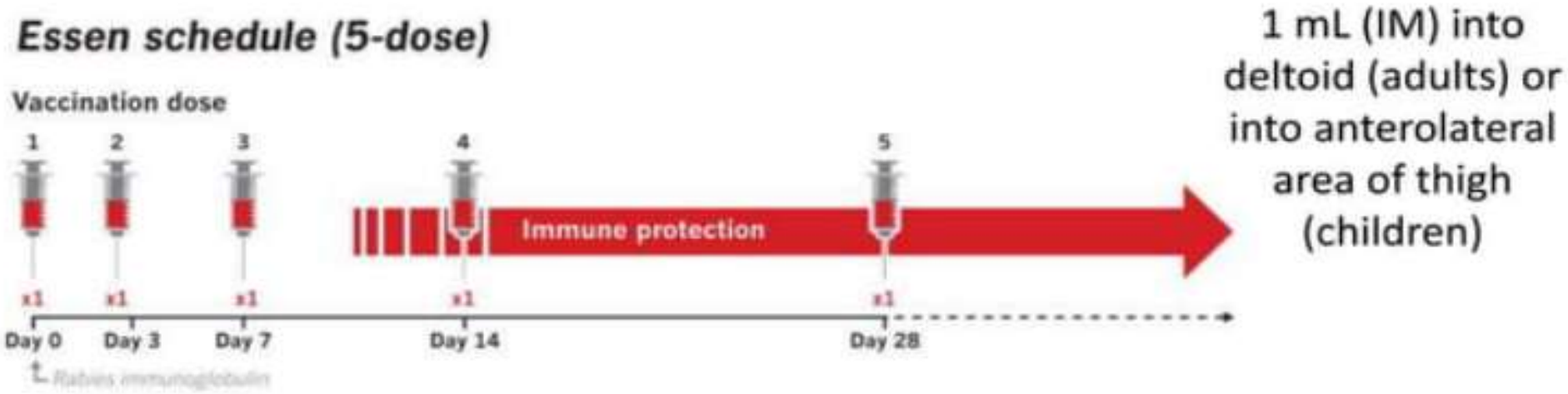
- Lasting immunological memory with CCVs & PDEVs
- Good anamnestic responses for booster vaccination in those who received primary series 5-21 years back
- No difference in response with route of vaccination
- Anti-rabies neutralizing antibody titre of 0.5 IU/ml or more in serum is considered as protective
- Achieved in most healthy individuals by day 14 of a post-exposure regimen

Common PEP Regimens

Regimen	Doses in the regimen	Site of injection	Amp used	Dose (ml)
Essen (IM)	1-1-1-1-1	Deltoid Ant Lat Thigh	5	0.5 or 1
Abbreviated Multisite IM (Zagreb)	2-0-1-0-1	Rt arm^{0}, Left arm^{0} Deltoid	4	0.5 or 1
8-site ID	8-0-4-0-1-1	Deltoid, thigh, Supx, lower ant abd	<2	0.1
Thai Red Cross (ID)	2-2-2-0-1-1	Both Deltoid	<2	0.1
Updated Thai Red Cross (ID)	2-2-2-0-2	Both Deltoid	<2	0.1

Post exposure prophylaxis IM administration Essen regimen

One IM dose of vaccine on Days 0, 3, 7, 14, and 28



1 mL (IM) into deltoid (adults) or into anterolateral area of thigh (children)

5 doses – 5 visits

RIG is always recommended for transdermal wounds

1. WHO 2004; 2. WHO 2007

Post exposure prophylaxis ID administration updated thai red cross (2-2-2-0-2)

Days 0, 3, 7, and 28 – two 0.1 mL doses

left deltoid

8 doses – 4 visits

RIG is always recommended for transdermal wounds

1. WHO 2004; 2. WHO 2007

ID Injection technique

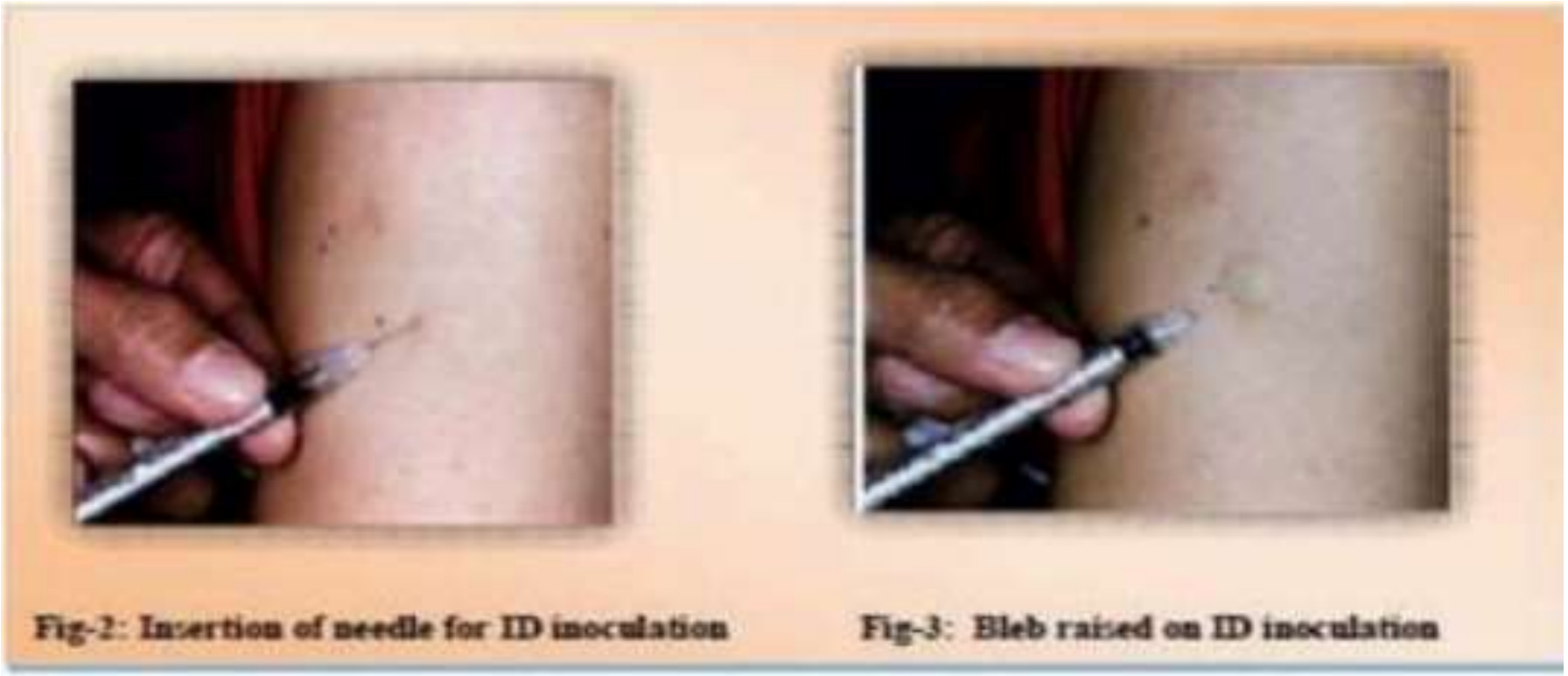


Fig-2: Insertion of needle for ID inoculation

Fig-3: Bleb raised on ID inoculation

- The needle should be almost parallel with the skin surface and the bevel of the needle facing upwards
- Inserted approximately 2 mm into the superficial layers of the dermis
- Intradermal injections reduce the volume of vaccine required and vaccine cost by 60% to 80% ID injection technique

DCGI recommended post-exposure IM and ID regimens : summary

Regimen	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	Day 90	Vials	Visits
Essen	1.0 mL	1.0 mL	1.0 mL	1.0 mL	–	1.0 mL	–	5	5

Regimen	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	Day 90	mL	Visits
Thai Red Cross (updated)	2 x 0.1 mL	2 x 0.1 mL	2 x 0.1 mL	–	–	2 x 0.1 mL	–	<1	4

Management of re-exposure in previously vaccinated individuals

- Priming of immune system and the development of immunological memory→ long lasting immunity
- Anamnestic response to one or more booster doses in persons previously receiving complete PrEP or PEP
- Two booster doses IM (0.5ml/1ml) or CCVs ID (0.1 ml at 1 site) on days 0 and 3
- Proper wound toilet should be done
- Treatment with RIG not required
- Persons previously receiving NTV or vaccines of unknown efficacy should be treated as fresh case & given full regimen

Pre – exposure prophylaxis (PrEP)

- Full IM dose or 0.1 ml ID dose to be given on days 0, 7 and either day 21 or 28
- Laboratory staff handling the virus and infected material
- Clinicians, Persons attending to human rabies cases
- Veterinarians, Animal handlers and catchers
- Wildlife wardens
- Quarantine officers and travelers from rabies free areas to rabies endemic areas
- Antibody titres checked every 6 months during the initial two years period after the primary vaccination
- If it is less than 0.5 IU/ml a booster dose of vaccine should be given. Subsequently, sero-monitoring is recommended every two years.

First & only person to survive rabies without vaccine

RABIES

Zero deaths by 2030

99% human cases result from **dog bites**

One death every **9 minutes** worldwide

4 out of 10 deaths are in **children**

THANK YOU

VACCINATE TO STOP TRANSMISSION

VACCINATE TO SAVE LIVES

100% vaccine preventable

no bite no rabies

World Health Organization

#rabies

28 September

World Rabies Day

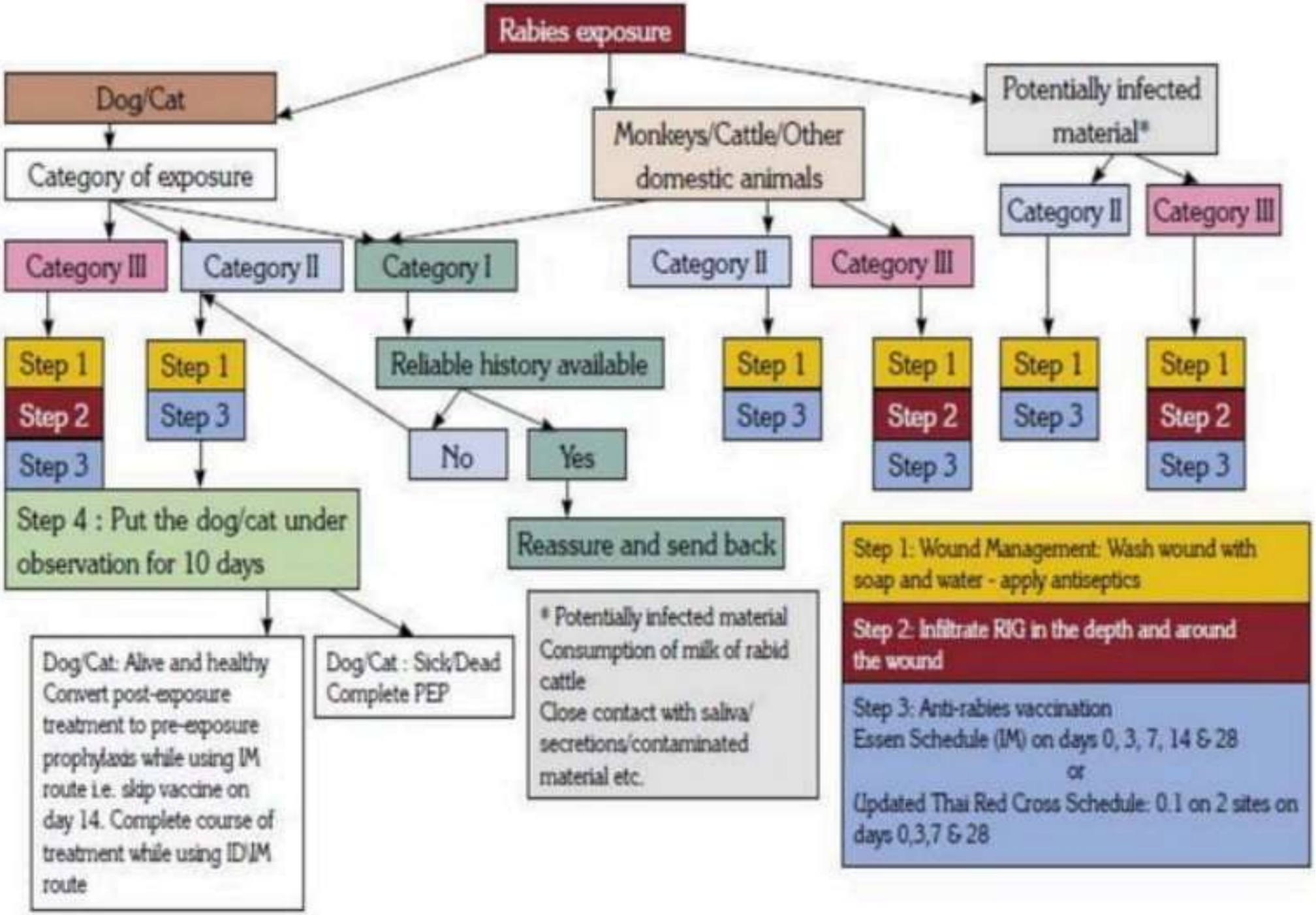

Rabies exposure
Dog/Cat
Category of exposure
Category III
Category II
Category I
Step 1
Step 2
Step 3
Step 1
Step 3
Step 4 : Put the dog/cat under observation for 10 days
Dog/Cat: Alive and healthy
Convert post-exposure treatment to pre-exposure prophylaxis while using IM route i.e. skip vaccine on day 14. Complete course of treatment while using ID/IM route
Dog/Cat : Sick/Dead
Complete PEP
Reliable history available
No
Yes
Reassure and send back
Monkeys/Cattle/Other domestic animals
Category II
Category III
Step 1
Step 3
Step 1
Step 2
Step 3
Potentially infected material*
Category II
Category III
Step 1
Step 3
Step 1
Step 2
Step 3
* Potentially infected material
Consumption of milk of rabid cattle
Close contact with saliva/ secretions/contaminated material etc.
Step 1: Wound Management: Wash wound with soap and water - apply antiseptics
Step 2: Infiltrate RIG in the depth and around the wound
Step 3: Anti-rabies vaccination
Essen Schedule (IM) on days 0, 3, 7, 14 & 28
or
Updated Thai Red Cross Schedule: 0.1 on 2 sites on days 0,3,7 & 28

Table 1 **Post-exposure prophylaxis (PEP) by category of exposure**
Tableau 1 **Prophylaxie post-vaccinale par catégorie d'exposition**

	Category I exposure – Exposition de catégorie I	**Category II exposure – Exposition de catégorie II**	**Category III exposure – Exposition de catégorie III**
Immunologically naive individuals of all age groups – Individus jamais encore immunisés quel que soit le groupe d'âge	Washing of exposed skin surfaces. – Lavage des surfaces cutanées exposées. No PEP required. – Aucune PPE n'est requise.	Wound washing and immediate vaccination: – Lavage de la plaie et vaccination immédiate: • 2-sites ID on days 0, 3 and 7[73] – Injections ID en 2 sites aux jours 0, 3 et 7[73] **or – ou** • 1-site IM on days 0, 3, 7 and between day 14–28[74] – Injections IM en 1 site aux jours 0, 3, 7 et entre 14 et 28 jours[74] **or – ou** • 2-sites IM on days 0 and 1-site IM on days 7, 21[75] – Injections IM en 2 sites au jour 0 et en 1 site aux jours 7, 21[75] RIG is not indicated. – L'immuno-globuline antirabique n'est pas indiquée.	Wound washing and immediate vaccination – Lavage de la plaie et vaccination immédiate • 2-sites ID on days 0, 3 and 7[73] – Injections ID en 2 sites aux jours 0, 3 et 7[73] **or – ou** • 1-site IM on days 0, 3, 7 and between day 14–28[74] – Injections IM en 1 site aux jours 0, 3, 7 et entre 14 et 28 jours[74] **or – ou** • 2-sites IM on days 0 and 1-site IM on days 7, 21[75] – Injections IM en 2 sites au jour 0 et en 1 site aux jours 7, 21[75] RIG administration is recommended. – L'administration d'immunoglobuline antirabique est recommandée.
Previously immunized individuals of all age groups – Individus précédemment immunisés quel que soit le groupe d'âge	Washing of exposed skin surfaces – Lavage des surfaces cutanées exposées. No PEP required. – Aucune PPE n'est requise.	Wound washing and immediate vaccination:* – Lavage de la plaie et vaccination immédiate:* • 1-site ID on days 0 and 3 – Injections ID en 1 site aux jours 0 et 3 **or – ou** • At 4-sites ID on day 0 – Injections ID en 4 sites au jour 0 **or – ou** • At 1-site IM on days 0 and 3 – Injections IM en 1 site aux jours 0 et 3 RIG is not indicated. – L'immunoglo-buline antirabique n'est pas indiquée.	Wound washing and immediate vaccination:* – Lavage de la plaie et vaccination immédiate:* • 1-site ID on days 0 and 3 – Injections ID en 1 site aux jours 0 et 3 **or – ou** • At 4-sites ID on day 0 – Injections ID en 4 sites au jour 0 **or – ou** • At 1-site IM on days 0 and 3 – Injections IM en 1 site aux jours 0 et 3 RIG is not indicated. – L'immunoglobuline antirabique n'est pas indiquée.

* Immediate vaccination is not recommended if complete PEP already received within <3 months previously. – La vaccination immédiate n'est pas recommandée si une PPE complète a déjà été reçue depuis <3 mois.

ID: intradermal injection; IM: intramuscular injection; RIG: rabies immunoglobulins. – ID: injection intradermique; IM: intramusculaire.

Treatment

- There is no specific treatment for rabies , it is invariably fatal.
- Most deaths are caused by temporary brain dysfunction with little to no damage occurring to the brain itself
- Willoughby et al at Children's Hospital of Wisconsin put Jeanna Giese into an induced coma with ketamine & midazolam ("Milwaukee protocol")
- Amantadine & Ribavarin was given
- Giese brought out of coma after 6 days once immunity regained

Prevention of human rabies

Magico-Religious Practices *(e.g. witchcraft, turmeric powder etc.) DO NOT HELP*

Wash the wound thoroughly with plenty of water and soap

Apply an antiseptic (povidone iodine) or even alcohol

Do not cover or Suture the wound

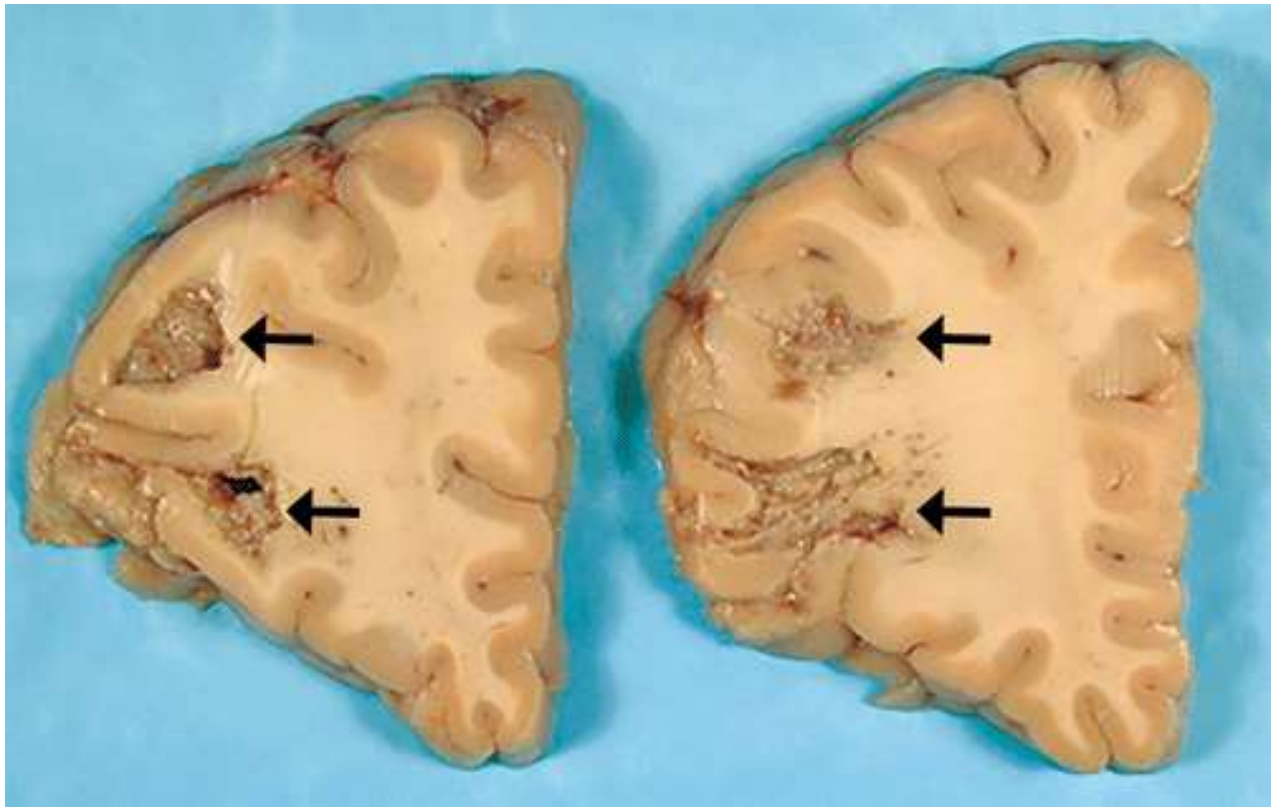
Vaccinate Immediately *e.g. Raibipur 1 mL*

RABIES

- Dr. R.G.W. Pinto, Professor & H.O.D Pathology, Ex-Dean Goa University
- Dr. Abarna.P, JR 1 Pathology

GROSS FEATURES

- Gross examination of brain shows intense edema and vascular congestion.

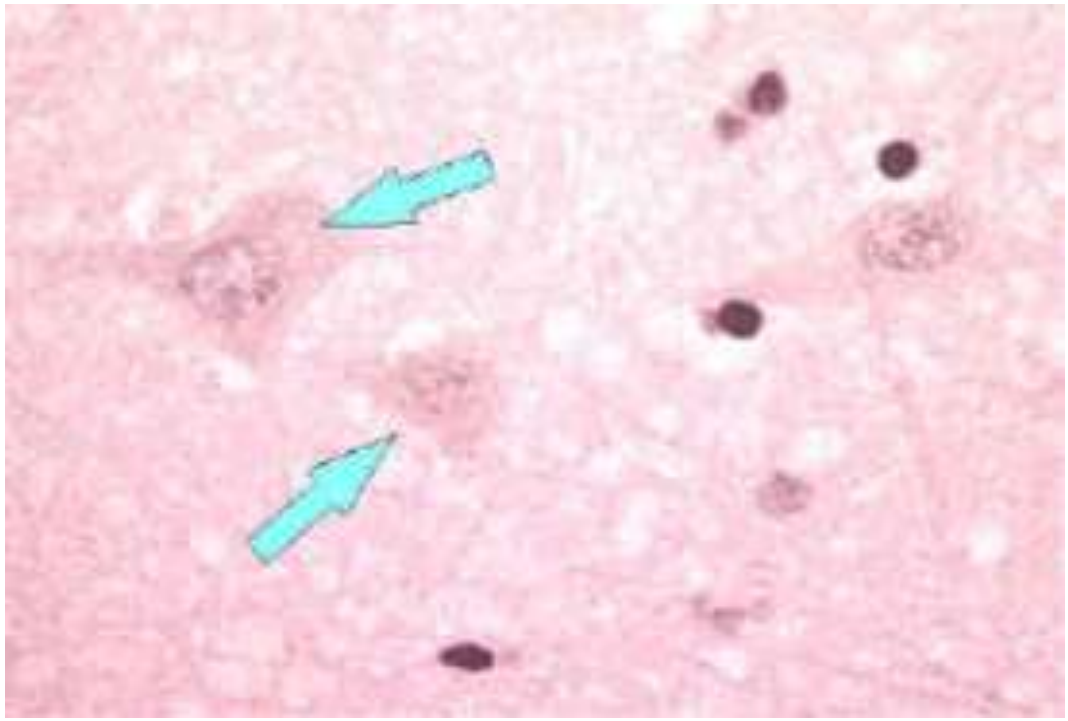


MICROSCOPIC FEATURES

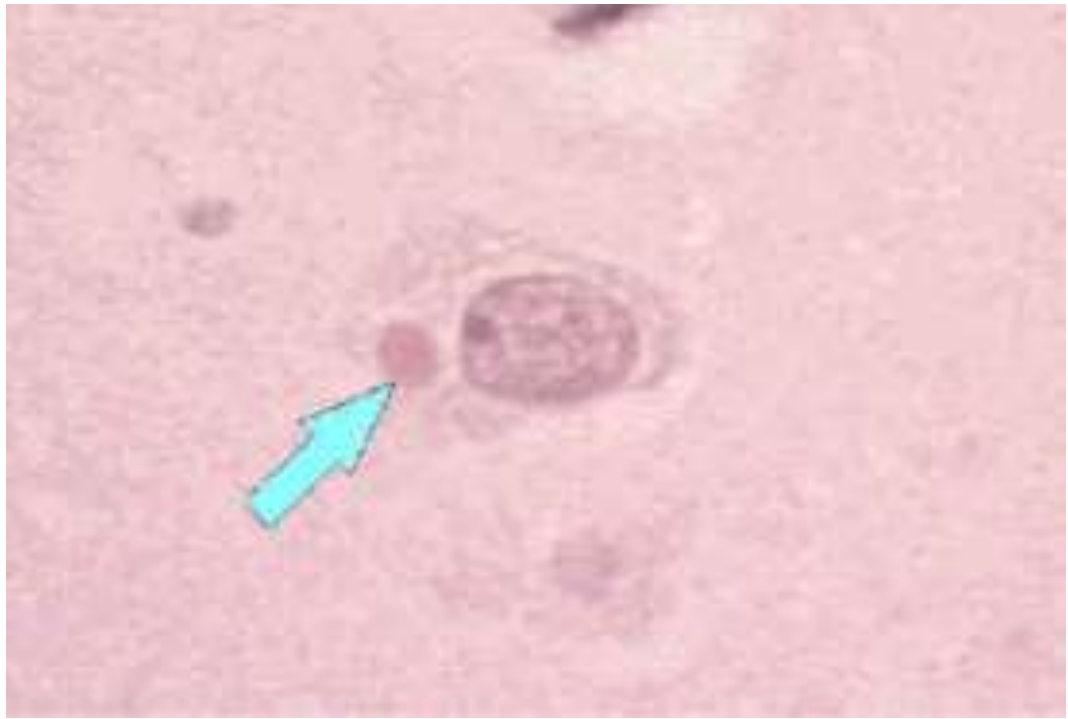
- Microscopically there is widespread neuronal degeneration and the inflammatory reaction which is most severe in brainstem, basal ganglia, spinal cord and dorsal root ganglia.
- *NEGRI BODIES* ,(the pathognomonic microscopic finding) are cytoplasmic, round to oval ,eosinophilic inclusions found in pyramidal neurons of the hippocampus and Purkinje cells of cerebellum ,sites usually devoid of inflammation.

Negri bodies :

- Normal neuronal cell

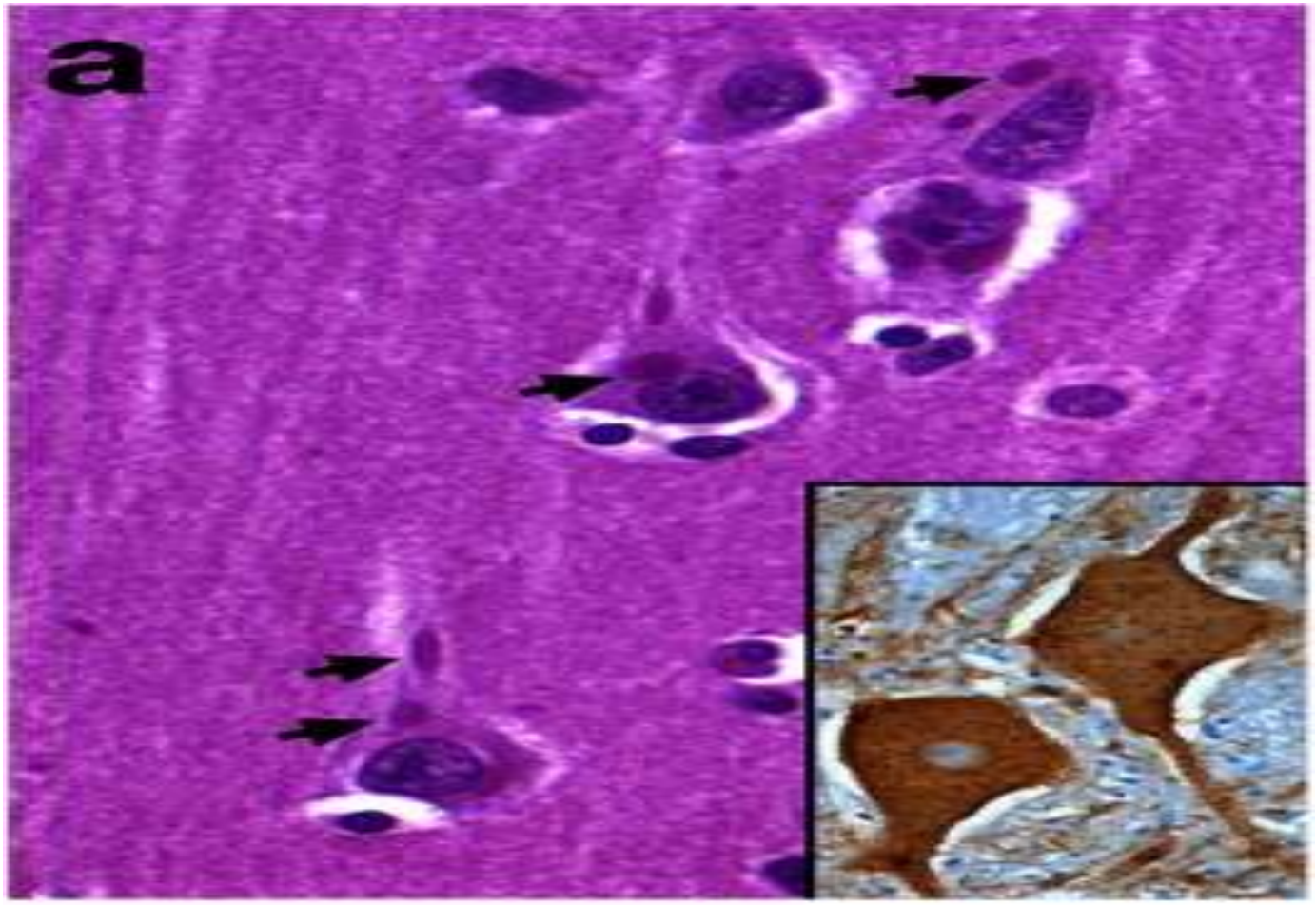


- Negri body in infected neuron



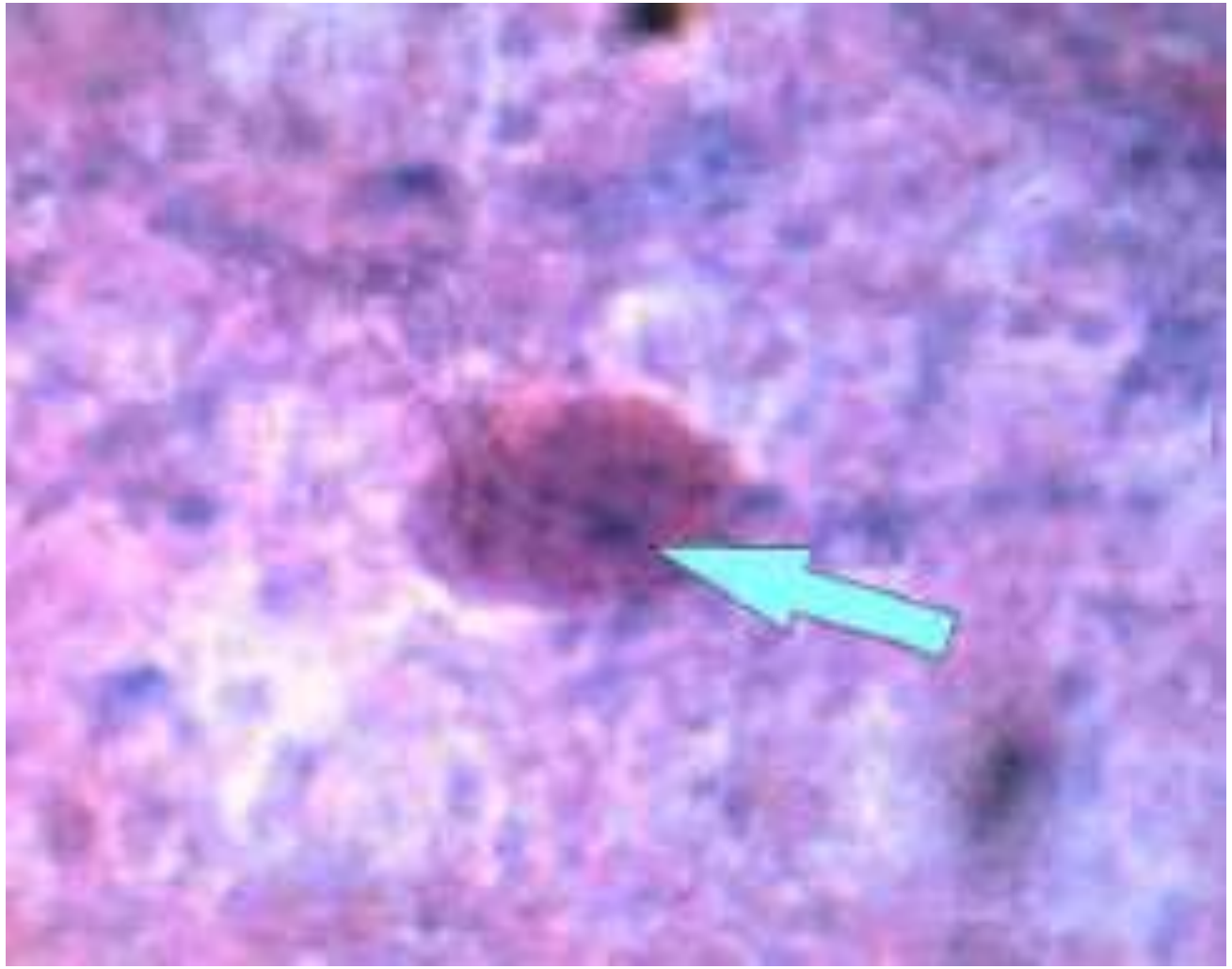
H&E STAINING :

- Pyramidal neurons of cerebral cortex showing multiple Negri bodies.
- Immunoperoxidase staining : rabies viral antigen seen as fine stippled deposits in the cytoplasm of neurons.



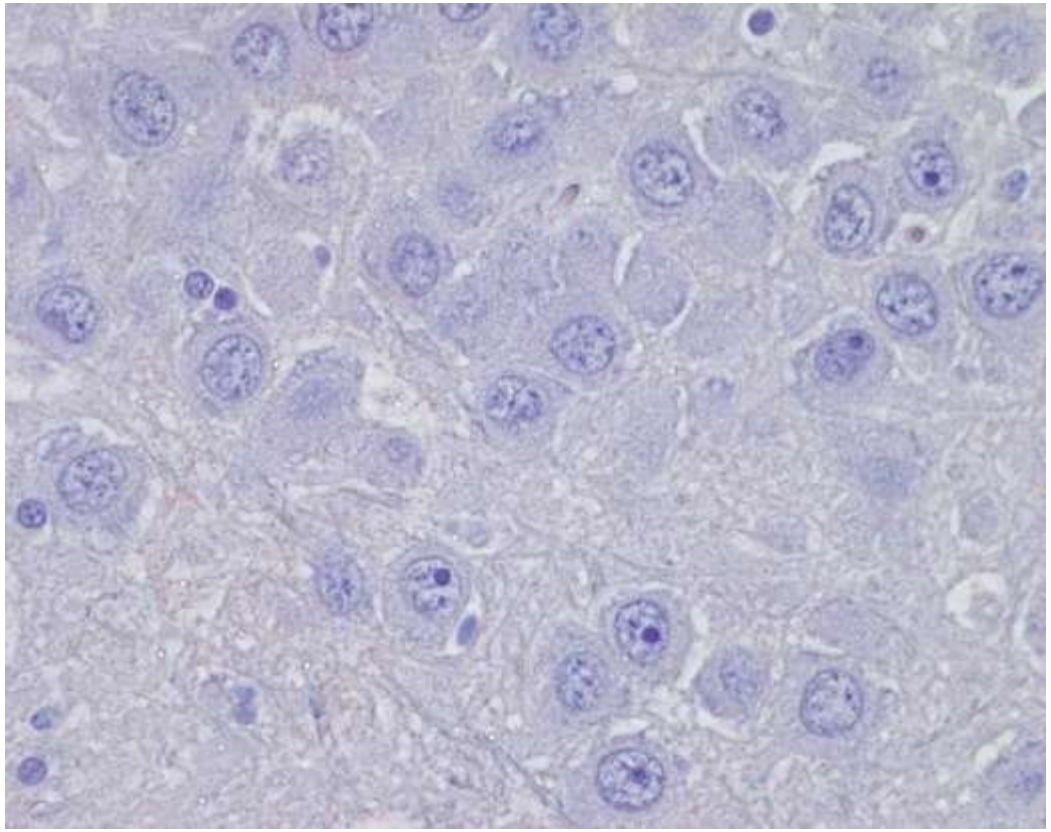
SELLERS STAIN :

- Enlargement of a Negri body in a Seller stained brain tissue.
- Basophilic inclusions (dark blue granules noted)

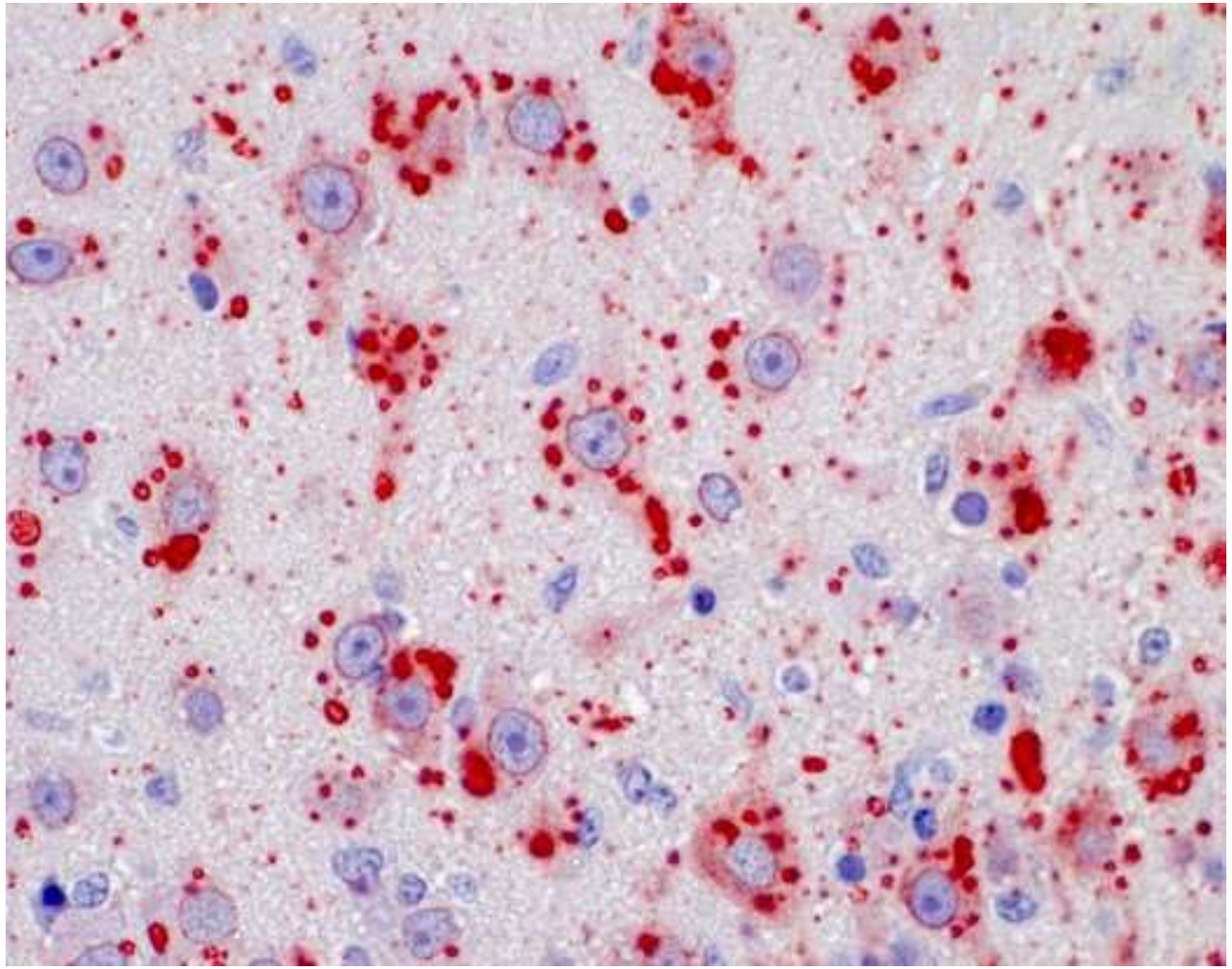


IHC :

- Brain tissue testing negative for rabies by IHC method ;no rabies virus antigen was detected.



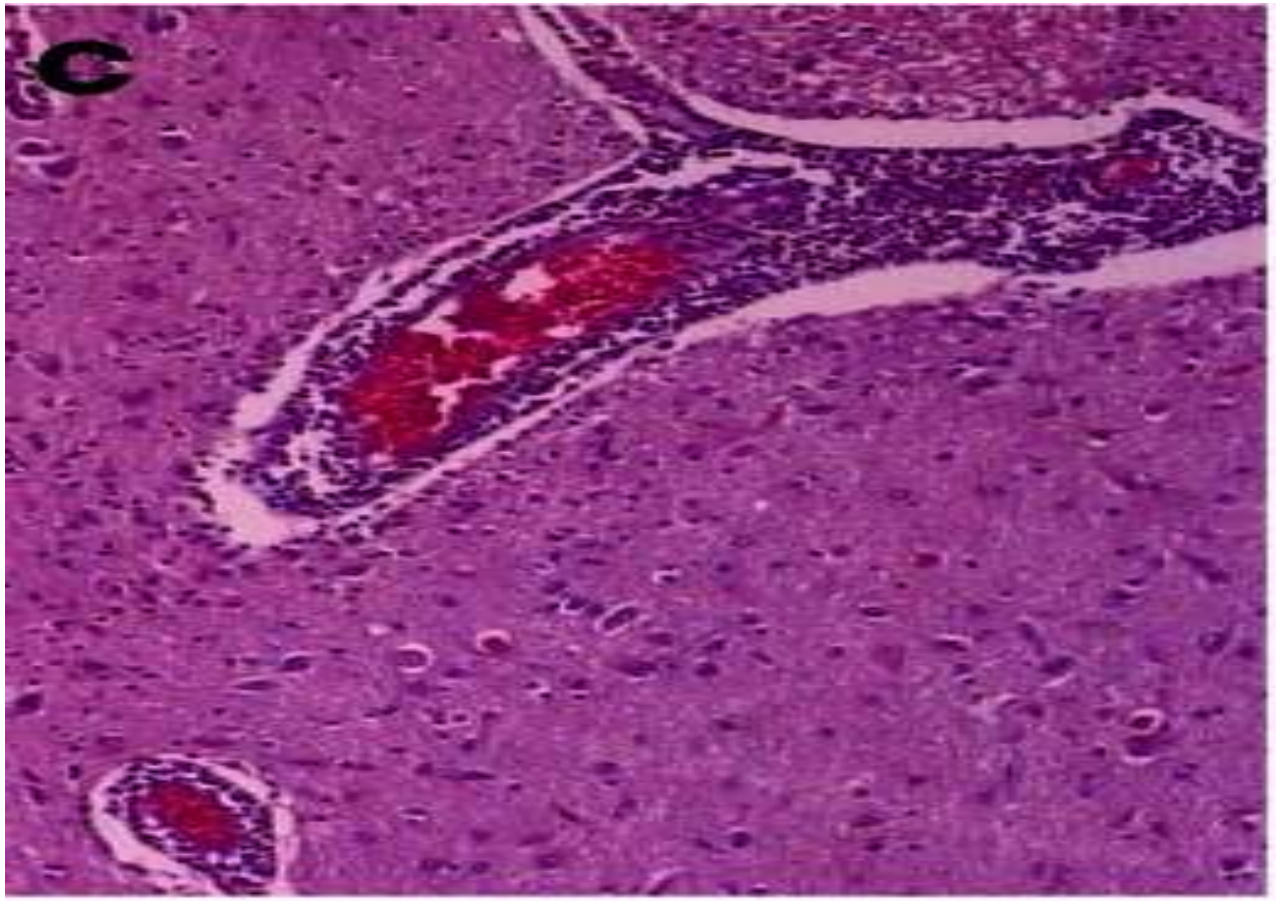
- Rabies infected neuronal cells of brain with intracytoplasmic inclusions.
- The red stain indicates presence of rabies virus antigen; using streptavidin biotin complex staining method.



RABIES ENCEPHALOMYELITIS :

- Histopathologic evidence of rabies encephalomyelitis in brain tissue and meninges include the following :
  - Mononuclear infiltration
  - Perivascular cuffing of lymphocytes or polymorphonuclear cells
  - Lymphocytic foci
  - Babes nodules consisting of glial cells
  - Negri bodies.

PERIVASCULAR LYMPHOCYTIC CUFFING :



BABES NODULES