Rabies virus

Dr Shyamal Kumar Paul

Associate Professor
Microbiology, MMC
An history of rabies

- Sanskrit “rabhas” - to do violence
- Celsus described hydrophobia 100AD

1885 Rabies vaccine – Dr. Louis Pasteur
Pasteur’s vaccine

- Grew virus in lab
  - lab strain lost ability to cause disease
  - this “live” virus used to innoculate first human subject, Joseph Meister in 1885
  - wasn’t purified - large number of injections
    - “shots” in the belly

- Vaccine development later improved...
World Rabies Day, Sep 8
Why is learning about rabies important?

- Rabies is 100% preventable yet at least 55,000 humans die from rabies each year around the world, mostly from exposure to dogs.
- A person dies of rabies almost every 10 minutes.
- Almost half of all rabies deaths occur in children under the age of 15 years.
- Many developed countries have vaccines for animals and humans to prevent rabies. These countries might only have a few deaths each year.
Rabies Virus

• Belongs to the genus Lyssavirus (lyssa: rage in Greek)
• Include members of the Rabdoviridae family: Rabies,
• Enveloped bullet-shaped virus
• 5 structural proteins
• - SS RNA, non-segmented, non-polar
Rabies virus

From CDC website: www.cdc.gov/ncidod/dvrd/rabies
Human rabies cases worldwide

Worldwide number of human rabies deaths/year est. 55,000
Most cases in India (> 30,000)
Rabies Virus

• Envelope contains G-protein spikes, which bind to cells

• Nucleocapsid core: Matrix (M) protein, viral nucleoprotein (N), viral RNA

• Transcriptase (L) protein, non-structural protein (NS)
Protein | Mol. wt. | Function
---|---|---
L | 244,000 | RNA-dependent RNA polymerase
G | 64-68,000 | Glycoprotein surface antigen
N | 55,000 | RNA-binding protein
NS | 40,000 | Phosphoprotein
M | 25-26,000 | Membrane/matrix protein

Lipid bilayer (host)
Rabies/Vector transmission

• **Spill over**: Rabid animals transmit rabies among same & other species
• **Compartmentalisation Concept**: specific virus variants within a genotype perpetuate among particular hosts in different geographic areas
• **Localized viral evolution**: geographic barriers
• **Occasional**: emergence of viral variants with extended host range
• STREET VIRUS  

Long IP  
• Muscle, Conective tissue and Nerve cell. Nerve cell  
• Negri body Present````  

Short IP  
Absent
Rabies/Vector transmission

• The dog is the most common cause of Rabies transmission worldwide, Cats 2nd
• In developed countries: dogs immunized, other species of wild animals are reservoirs
• Bats: always considered rabid
• In the past: < 10% of animal rabies in USA and Canada

Variants of bat rabies virus has become the most common cause of rabies death
Rabies surveillance in animals/USA

- > 92% wild animals, 7.4% domestic species
- Raccoons: 36.3% most common
- Skunks: 30.5%
- Bats: 17.2%
- Foxes: 6.4%
- Cats: 3.8%
- Dogs: 1.2%

Rabies/Vector transmission

- Caribbean: Mongoose
- Europe: Red fox
- Iran: Wolf
- Africa: Jackal

Hammond GW (Principles and Practice of Pediatric Infectious diseases)
Global distribution of mammalian rabies reservoirs and vectors
A productive pathogenesis cycle of animal rabies: virus entry into peripheral nerves via a bite, movement to the central nervous system resulting in encephalitis, and transit to the salivary glands, mediating infection of another host. 

Rupprecht CE et al, The Lancet Infectious Diseases Vol 2 June 2002
Rabies attacks the Central Nervous System

- Watch as the rabies virus from an exposure on the leg spreads up the spinal cord to the brain and throughout the rest of the body.

Rabies virus entering the body.
Foxes maintain rabies from Arctic areas to temperate and tropical latitudes
Gray fox: A surge of rabies cases among gray foxes in Texas in 2002
The Jackal is an important candid reservoir of rabies in the old world.
Mongoose and related species are important in parts of Africa, Asia & the Caribbean. Transported from Asia for snake control in sugar-cane plantations.
Rabid wolves are associated with severe bites and human deaths. Wolves may not serve as true rabies reservoirs.
Hosts 6/7 lyssavirus genotypes

Widespread throughout North America, Latin America

Infection rates in bats varies (4% to > 15%)

Humans encounter bats that are sick, incapacitated

Different bat species vary in their human interaction

Primary reservoir for rabies in All continents.

1/4/2014

Dr. Shyamal Kr Paul, Rabies virus
Rabies/Bats

- At least 39 cases in USA
- Only 9 (23%) has hx. of bite
- 20 (51%): known or likely contact with bats
- Bite is most likely mode of transmission
- Bat rabies viruses vary in their virulence properties
- Minor lesions should not be ignored

Rupprecht CE et al, The Lancet Infectious Diseases Vol 2 June 2002

1/4/2014 Dr. Shyamal Kr Paul, Rabies virus
Rabies/Dogs

- IP: usually < 10 days
- May be one year
- Change in disposition, restlessness, fear
- “Furious” or “dumb” syndrome
- Death within 10d of symptoms
- Wild animals: similar symptoms, lack of fear of man

Hammond GW (Principles and Practice of Pediatric Infectious diseases)
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Dr. Shyamal Kr Paul, Rabies virus
Rabies/Vector transmission
A rabid dog displaying the classic form of paralytic rabies, including cranial-nerve deficits and hypersalivation

Rupprecht CE, The Lancet Infectious Diseases Vol 2 June 2002
Rabies/transmission

- Infected animal saliva inoculated by bite or scratch
- Infected saliva: contact with mucous membrane, transdermal exposure
- Virus shed in the saliva during, before or after clinical symptoms
- Human-Human: few reported cases following corneal transplantation
- Aerosol transmission: caves containing bats, lab work accident
Rabies/Pathogenesis

• Risk of acquisition:
  • bite 5–80%
  • Scratch 0.1–1.0%
• Lyssavirus genotype dependent
• Dog: Nicotinic acetylcholine receptor on muscle
• Bat: Unknown receptor on epidermis/dermis
• Skunks: rabies virus antigens and genome can persist for months in muscle: host clearance, treatment
Rabies/Pathogenesis

- Budding from the plasma membrane of muscle cells into unmyelinated nerve endings
- Retrograde axoplasmic flow to the CNS
- Virus replication in dorsal root ganglia (DRG) and anterior horn cells
- Immune response to virus in DRG: neuropathic pain (Bat > dog)
- Prophylaxis at this stage cannot prevent death
Rabies/Pathogenesis

- Direct access of virus to peripheral nerves
- Travel to CNS at rate of 8-20mm/day
- Neuromuscular junction is the major site of entry into neurons
- Receptors on nerves that are used by the virus: Nicotic acetylcholine, neural adhesion molecule (CD56), NGF (p75 neurotrophin) receptor
- Viral spread to other neural cells via G-protein
Rabies/Pathogenesis

CNS infection

- Virus reaches CNS: rapid dissemination
- Preferential localization in brain stem, thalamus, basal ganglia, spinal cord
- Clinical manifestations of rabies are not totally explained by host, viral strain, virus localization
- Development of paralytic rabies is more likely after bite by vampire bat
- Paralytic rabies may have genetic predisposition
Rabies/Pathogenesis
CNS infection

- Eventually, the virus spreads centrifugally from the CNS to the heart, skin, salivary and serous glands in the tongue
- All major organs may contain the virus (except blood)
- Organs from patients with unexplained neurologic disease may transmit rabies by transplantation

Hemachudha T., The Lancet Neurology Vol 1, June 2002
1/4/2014 Dr. Shyamal Kr Paul, Rabies virus
Rabies/clinical manifestations

- Most cases are males < 15yr
- 4 phases of illness
- First phase: asymptomatic
- Virus IP: 10–90 days (4d–19yr)

Hammond GW (Principles and Practice of Pediatric Infectious diseases)
Rabies/clinical manifestations

- Second (prodromal) phase
- 2–10d
- Viral invasion of CNS (limbic system, spinal cord, brain stem)
- Respiratory symptoms
- Gastrointestinal symptoms
- Behavioral & emotional symptoms
- Local pain itching, numbness (50%)
Rabies/clinical manifestations

• Third phase: neurologic signs
• Widespread infection of the brain
• “Furious”:
  
  Hyperactive form
  Aggressiveness, biting, yelling, hallucinating
  Triggered by sensory stimuli
  Hydrophobia: drinking liquids
  Aerophobia: air blown on face
  Violent diaphragmatic contractions
  Hyper-reflexia, cholinergic manifestations
  Lacrimation, salivation, mydriasis, pyrexia
Non-Classical Rabies/clinical manifestations

- Most commonly after Bat exposure
- Bat rabies is different from dog rabies
- Third phase: neurologic signs
- "Paralytic" form: 20% of patients
- Flaccid paralysis and paresis
- Mimics GBS, transverse myelitis
- Inflammation is more extensive and severe
- Spinal cord markedly involved

Hammond GW (Principles and Practice of Pediatric Infectious diseases)
1/4/2014
Dr. Shyamal Kr Paul, Rabies virus
Non-Classic Rabies/clinical manifestations

• Neuropathic pain, radicular pain, objective sensory and motor deficits
• Choreiform movements of the bitten limb during prodromal phase
• Focal brain stem signs, myoclonus
• Hemiparesis, hemisensory loss, ataxia, vertigo, Horner’s syndrome
• Seizures, ataxia
Non-Classic Dog Rabies/clinical manifestations

- Ocular myoclonus, hemichorea
- Nocturnal agitation
- Repeated spontaneous ejaculation (autonomic dysfunction)
- Paraparesis
- Facial & pulbar weakness
- Bilateral arm weakness
- Seizures, ataxia
Rabies/clinical manifestations

Both forms:
- Fever
- Nuchal rigidity
- Paresthesia
- Fasiculations
- Convulsions
- Hypersalivation
- Hyperventilation
Rabies/clinical manifestations

- Fourth phase: Coma
- Extensive cortical virus spread
- Death usually in 7 days
- Respiratory arrest
- Myocarditis
- Supportive care: sedation and analgesia

Hammond GW (Principles and Practice of Pediatric Infectious diseases)
Rabies/Recovery

- Rare survivors
- Atypical presentations
- 1972: bat related, unsteady gait, dysarthria, hemiparesis
- 1976: dog bite, quadreparenesis, myoclonus, cerebellar signs, frontal lobe signs
- 1977: Lab worker, aerosol exposure to highly concentrated fixed rabies virus
- 1992–1995: 4 Mexican children (3: dog, 1: vampire bat), received vaccine, no Ig
Rabies/clinical manifestations

- Mortality depends on
  - Severity of injury: bleeding
  - Location of the wound: face, head, neck, hand: short IP
  - Virus conc. in saliva
- Rabies mortality of untreated bite by rabid dog: 38-57%
- Rabid wolves: MR 80%
- Rabid bats: risk even with superficial wound (replication of virus in epidermis/dermis)
Rabies/Diagnosis/Culture

- **Viral culture**: skin biopsy of the hair follicles at nape of the neck
- **Virus culture**: saliva, CSF, urine, respiratory secretions
- **Culture in mice or in mouse neuroblastoma cell line**
- **Sensitivity 50-94%, specificity 100%**

Hammond GW (Principles and Practice of Pediatric Infectious diseases)
Diagnosis/Tissue studies

- Brain tissue: culture, histology for Negri bodies: yield low
- Immunohistochemistry on tissue
- Brain tissue: Immunostain (higher yield)

Hammond GW (Principles and Practice of Pediatric Infectious diseases)
Rabies/Diagnosis

- Rabies specific antibodies in serum or CSF (RFFIT)
- Serology positive in serum in 7 days of symptoms
- Serology positive in CSF in 13 days of symptoms
- Rabies vaccine does not cause positive CSF antibodies
- Molecular studies, monoclonal antibodies in epidemiologic studies
Section of rabid human brain processed by the DFA test, showing widespread viral inclusions, staining apple-green in colour.

Rupprecht CE, The Lancet Infectious Diseases Vol 2 June 2002
A neuron from a formalin-fixed section of a brain from a patient with rabies, showing reddish-brown viral inclusions in the cytoplasm. Processed by immunohistochemistry.
Immunofluorescent viral inclusions in a peripheral nerve in a cryostat section from a patient with rabies, obtained via an antemortem nuchal skin biopsy.

Rupprecht CE, The Lancet Infectious Diseases Vol 2 June 2002
Rabies/Prevention

- Pre-exposure prophylaxis: vaccination of people in high risk groups:
  - Veterinarians
  - Animal handlers
  - Certain lab workers
  - Travel to areas where canine rabies is common

Hammond GW (Principles and Practice of Pediatric Infectious diseases)
Rabies/Prevention

- Pre-exposure prophylaxis: vaccination: intramuscular, 1ml (3 doses): at 0, 7, 21-28 days
- Antibodies usually persist for 2 yrs
- Repeat titers q6-24 months depending on level of exposure
- Acceptable titer levels are 1:5 or 0.5 IU/ml (RIFFT)

Red Book 2003
Rabies/Prevention

vaccine types

• Human Diploid Cell Vaccine (HDCV)
• Rabies Vaccine adsorbed (RVA)
• Purified chicken embryo cell (PCEC)
Rabies/Post-exposure prophylaxis

- Consult local health department
- Type of animal bite
- Unprovoked attack vs a bite during attempt to feed or handle the animal
- Immunized animals: minimal risk
- Prophylaxis to anyone bitten by
  - wild mammalian carnivores
  - bats
  - potentially infected domestic animals
### Animal type, assessment, and disposition

<table>
<thead>
<tr>
<th>Animal type, assessment, and disposition</th>
<th>Recommended treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog, cat, ferret</td>
<td></td>
</tr>
<tr>
<td>Healthy and available for 10 days observation</td>
<td>None unless the animal develops signs of rabies; the animal should then be killed and tested</td>
</tr>
<tr>
<td>Rabid or suspected rabid</td>
<td>Start postexposure prophylaxis</td>
</tr>
<tr>
<td>Unknown (eg, escaped)</td>
<td>Consult public-health officials</td>
</tr>
<tr>
<td>Skunks, raccoons, foxes, and most other carnivores; bats</td>
<td>Regard as rabid until proven negative by laboratory test</td>
</tr>
<tr>
<td>Livestock, small rodents, lagomorphs (rabbits and hares), and other mammals</td>
<td>Consider immediate vaccination</td>
</tr>
<tr>
<td>Consider individually</td>
<td>Consult public health officials: bites of squirrels, hamsters, guineapigs, gerbils, rats, mice, and other small rodents almost never require postexposure prophylaxis</td>
</tr>
</tbody>
</table>
Rabies/Post-exposure prophylaxis

• Exposure other than bite rarely causes infection

• Prophylaxis to patients with
  • open wound
  • scratch
  • mucous membrane contaminated by
  • saliva or
  • potentially infectious material from rabid animal
Rabies/Post-exposure prophylaxis

• Prophylaxis to patients with bat exposure if bite or mucous membrane exposure cannot be reliably excluded
  • Bat in a room with pt asleep
  • Bat in a room with unattended child

• No prophylaxis if bat caught and promptly tested negative
Rabies/Post-exposure prophylaxis

Humans with rabies

- Prophylaxis to people with sig. exposure to a rabies pt. if
  - scratch
  - bite
  - mucous membrane exposure to saliva or infectious tissue

- No prophylaxis if casual contact (touching) or exposure to non-infectious material (urine, stool)
Post-exposure wound care

- Prevent virus in wound from reaching neural tissue
- Prompt and thorough cleaning: flush wound with soap and water
- Benzalkonium chloride not superior to soap
- Update tetanus immunization
- Treat secondary bacterial infection
- Do not suture wound if possible
Post-exposure immunoprophylaxis

- Passive and active
- Start ASAP
- RIG and rabies vaccine
- Vaccine: one of the 3 types (5 doses), same dose for all ages
- 1.0 ml IM at 0, 3, 7, 14, 28 d
- Intradermal regimens: used in some countries, not USA
- Avoid gluteal injection: less antibody response than deltoid or AL thigh
Immunoprophylaxis/RIG

- Human RIG is Given at the same time with the vaccine (ASAP)
- Dose: 20 IU/kg
- As much as possible to infiltrate the wound
- Remainder is given IM
- RIG and vaccine are Give at different sites & in different syringes
- Purified equine RIG (outside USA): dose is 40 IU/kg, may need desensitization
Immunoprophylaxis/RIG contraindications

• Persons who received a 3-dose pre-exposure rabies vaccine

• Those with adequate antibody response after previous immunization: give 2 doses of vaccine at 0,3 days

• Those who received post-exposure prophylaxis with rabies vaccine (>7 d)
Rabies Vaccine
Adverse effects

• Less common in children than adults
• Adults: local rxn. (15-25%)
• Mild systemic rxn. (10-20%)
• Neurologic illness resembling GBS
• Acute generalized transient neurologic syndrome: not causally related
• Immune-complex reactions with booster doses of HDCV: 6%
Handling of suspected rabid animal

- Management depends on the species, the circumstances of the bite and local epidemiology of rabies
- **Dog, cat, ferret** with suspected rabies should be captured and observed for signs of illness x 10 days
- If ill: euthanatized, head removed and shipped for examination
- Species with unknown periods of viral shedding may still be euthanatized and tested even if immunized
Rabies/prophylaxis

- Bats, skunks, raccoons, foxes, most other carnivores:
- Regard as rabid unless geographic area is known to be free of rabies or until animal proven negative by lab testing
- Immediate immunization and RIG
Rabies/prophylaxis

- Livestock, rodents, and lagomorphs (rabbits & hare):
  - Consult local health department
  - Bites of squirrels, gerbils, hamsters, guinea pigs, rats, mice, other rodents, rabbits, hare almost never require anti-rabies treatment
Handling of suspected rabid animal

- Wild animals with suspected rabies should be euthanatized at once and brain tested for rabies.
- No treatment for rabies if animal brain tests negative by rapid test (fluorescent antibody testing).
Rabies prevention

• Educating children to avoid contact with stray or wild animals
• Avoid trying to capture or provoke stray animals
• Avoid touching animal carcasses
• Secure garbage
• Chimneys, other entrances should be covered
• International travelers: avoid contact with stray dogs, consider rabies vaccine
Post Exposure Prophylaxis/WHO

- **Category I:**
  - touching
  - feeding potentially rabid animal
  - licking intact skin
  **no treatment**

- **Category II:**
  - nibbling on uncovered skin
  - licks on broken skin
  - minor scratches without bleeding
  **wound disinfection, vaccine only**
Post Exposure Prophylaxis/WHO

- **Category III:**
  - Single, multiple transdermal bites
  - Contamination of scratches or MM with saliva
    wound cleansing, rabies IG, vaccine

- Animal observation in developing countries is not practical: frequent bites, delayed lab testing

- Delay treatment only if:
  - Species unlikely to be infected
  - Lab diagnosis in 48hr
  - Dog >1yr old with current vaccination (observe for 10d)
Prophylaxis/Nerve tissue vaccines

- Not licensed in USA, available worldwide
- Only available vaccines in some countries
- Nerve tissue from sheep, goats, suckling rodents, mouse brain
- Subcutaneously
- 7 daily doses, plus days 10, 20, and 90
Rabies Vaccine
nerve tissue vaccines

• Inactivated vaccines
• Neuroparalytic reactions in 1:2000 to 1:8000
• Discontinue if a neurologic reaction occurs
• Steroids for life-threatening reactions
Rabies Vaccine variations

• Attempts to reduce the cost of PEP
• Reduced IM regimen (2-1-1): 2 doses on day 0, 1 dose (day 7), 1 dose (day 21)
• Intradermal regimens
  • 8 site regimen: 8-0-4-0-1-1-1 (0.1ml doses): sites include both deltoids, lat thighs, lower quadrants of the abdomen, suprascapular areas
  • 2 site regimen: 2-2-2-0-1-1-1 (each=20% of IM dose): deltoids
Rabies post-exposure vaccination schedules for the rabies-naive patient

<table>
<thead>
<tr>
<th>Schedule</th>
<th>Days</th>
<th>0</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard WHO schedule(^a)</td>
<td></td>
<td>1 IM dose deltoid (^b)</td>
<td>1 IM dose deltoid</td>
<td>1 IM dose deltoid</td>
<td>1 IM dose deltoid</td>
<td>1 IM dose deltoid</td>
<td>1 IM dose deltoid</td>
<td>1 IM dose deltoid</td>
</tr>
<tr>
<td>Reduced multi-site IM (2-1-1)</td>
<td></td>
<td>2 IM doses; right and left deltoid</td>
<td>1 IM dose deltoid</td>
<td>1 IM dose deltoid</td>
<td>1 IM dose deltoid</td>
<td>1 IM dose deltoid</td>
<td>1 IM dose deltoid</td>
<td>1 IM dose deltoid</td>
</tr>
<tr>
<td>8 site ID regimen (8 0 4 0 0 0 1 1)</td>
<td></td>
<td>8 x 0.1 mL ID</td>
<td>4 x 0.1 mL ID</td>
<td>0.1 mL ID</td>
<td>0.1 mL ID</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 site ID regimen (2 2 0 0 0 2 1 1)</td>
<td></td>
<td>2 x 20% IM ID</td>
<td>2 x 20% IM ID</td>
<td>20% IM ID</td>
<td>20% IM ID</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suckling-mouse-brain vaccine(^c)</td>
<td></td>
<td>1 dose each subcutaneously on abdomen</td>
<td>1 dose</td>
<td>1 dose</td>
<td>1 dose</td>
<td>1 dose</td>
<td>1 dose</td>
<td>1 dose</td>
</tr>
</tbody>
</table>

\(^a\) Rupprecht CE et al, The Lancet Infectious Diseases Vol 2 June 2002

\(^b\) Deltoid = posterior deltoid:

\(^c\) Lobo A, Rupprecht CE, TVAC and VAC, The Lancet Infectious Diseases Vol 2 June 2002
Rabies Vaccine/
Future developments

• DNA vaccines:
  - Can expand lyssavirus cross-reactivity
  - Primary inoculation
  - A booster dose
• Recombinant vaccines
• Plant biotechnology for production of Ag
• Development of neutralizing monoclonal antibodies
Animal vaccination

- Several states initiated raccoon rabies programs
- Oral rabies vaccine delivered by baits
- Baits: polymer cubes (dog food or fish meal), wax-lard cake, attractants: fatty, cheesy, sweet odors
- Effective for coyotes and foxes
- Raccoons compete for baits
- Current oral vaccine is not effective for skunks

A raccoon consuming a bait laden with oral rabies virus vaccine

1/4/2014  Dr. Shyamal Kr Paul, Rabies virus
Rupprecht CE et al, The Lancet Infectious Diseases Vol 2 June 2002