RABIES

(Hydrophobia, Lyssa, Rage; □□□□□□ □□□)

Rabies is an acute, progressive, fatal, viral encephalomyelitis (inflammation of both brain and spinal cord), which affects all warm-blooded animals, including humans. Its a highly fatal disease, mortality rate being close to 100%. Globally, Dog is the most important reservoir.

The disease is transmitted by the bites of affected animals, most commonly of dogs and cats. The disease is endemic in wildlife, particularly foxes, wolves, mongoose, raccoons, and bats.

Cause

Rabies is a viral disease caused by a BULLET-Shaped **Lyssa virus** of **Rhabdovirus** family, which is truly neurotropic and causes lesions only in nervous tissue. Strains of rabies virus isolated from naturally occurring cases are referred to as "**street virus**" (*Negri body +nt ; Salivary gland affected*), and attenuated laboratory strains are referred to as "**fixed virus**" (*Negri body -ve ; Salivary gland affected*).

Spread

Lyssaviruses are highly Neurotropic. Hematogenous spread does not occur. The source of infection is always an infected rabid animal, and the method of spread is almost always by the **Bite of a rabid animal**, and transmission **almost always occurs via introduction of virus-laden saliva into tissues.** Rarely, virus may be introduced through intact or abraded mucous membrane. Several factors, including (i) the virulence of the strain, (ii) quantity of infectious virus in the saliva, and (iii) the susceptibility of the species, play a role in establishing rabies in the recipient animal.

The rabies virus is maintained by interrelated cycle wherein the (i) Urban Cycle of rabies - is in dogs,cats, and (ii) Sylvatic Cycle of rabies-maintained in wild reservoirs like foxes, wolves, bats etc.

Traditionally, the dog, and to a minor extent the cat, fox, wolf, mongoose, bats etc have been considered to be the principal source of infection, which transmit the disease to cattle, horses, and sheep, which seldom spread it further. Multiplication of the virus without invasion of the nervous system is known to occur in fatty tissues in bats, and may be the basis of the 'reservoir' mechanism'. The virus may also appear in the milk of affected animals, but spread by this is not known to occur.

Pathogenesis

The incubation period is both prolonged and variable. Following a bite of rabid animals and after entry of rabies virus, there is a variable incubation period of **1 week to 1 year**, with a mean of 1-2 months. The length of the incubation period after viral exposure varies greatly and depends on (i) the anatomical distance between the bite site and the central nervous system, (ii) the severity of the bite, and (iii) the amount of infectious virus in the saliva.

Virus first replicates in the **myocytes (muscle cells) at the site of bite** and is shed into extracellular spaces. The virus **then enters the nervous system at motor end plates**, and **binds to the receptor for acetylcholine**, a neurotransmitter.

Following entry into the **peripheral or cranial nerves**, the virus spreads within the **axons of nerve cells** to the **ventral horn cells of the spinal cord** at a rate of 3-4 mm / hour. Virus replication occurs first in the spinal cord cells, and then it spreads to the brain, from where virus is disseminated throughout the central nervous system.

Direct transneuronal transfer of virus from neuronal perikarya (the cell bodies of neurons) and dendrites to adjacent axon terminals is a mechanism of dissemination of rabies in the central nervous system. **Brain stem, cerebral cortex, and hippocampus are particularly susceptible to rabies infection, and the destruction of neurons in these regions gives rise to clinical symptoms of rabies**.

There is **eventual CENTRIFUGAL SPREAD** (i.e., away from the centre, towards periphery) via nerves throughout the body to other tissues, **including the salivary glands**. Rabies virus can spread from the CNS to salivary glands, cornea, and tonsils, via their nerve supply. Viral replication in salivary gland occurs rapidly and infected saliva is the major source of infection. The only lesions produced are in the central nervous system, and spread from the site of infection occurs only by way of the peripheral nerves.

Bites on the head usually result in a shorter incubation period than bites on the extremities. The **severity** and the **sites of lesions** govern to a large extent, whether the clinical picture will be one of "**furious form**" or "**paralytic (dumb) form**". When irritation phenomena (induced by the virus in the nerve cells) occur, they are followed by paralysis as the stimulated nerve cells are subsequently destroyed. Destruction of spinal neurons results in paralysis and in the "paralytic (dumb) form" of the disease. But when the virus invades the brain, irritation of higher centres produces manias (madness), excitement, and convulsions. Death is usually due to respiratory paralysis.

Signs

The most reliable signs of Rabies, regardless of species, are acute behavioral changes and unexplained progressive paralysis. Behavioral changes may include sudden anorexia, signs of apprehension or nervousness, irritability, and hyperexcitability. The animal may seek solitude. Ataxia, altered phonation, and changes in temperament are apparent. The disease progresses rapidly after the onset of paralysis, and death is virtually certain within 10 days of the first signs.

The clinical course may be divided into three general phases—prodromal, acute excitative, and paralytic/endstage. However, this division is of limited practical value because of the variability of signs and the irregular lengths of the phases. The clinical symptoms usually appear in one of two forms:

1) "Dumb or Paralytic Form", and

2) "Furious Form".

In the "**Dumb or Paralytic Form**" of rabies , (i) the animal is in depressed, sulky condition, falls into a stupor (unconsciousness), and has a peculiar staring expression & an **unusual friendliness** can be seen. This paralytic form is characterized by (ii) early paralysis of the throat and muscles of mastication, usually with profuse salivation and inability to swallow. (iii) Dropping of the lower jaw is common in dogs. (iv) These animals are not vicious, and rarely attempt to bite. (v) The paralysis progresses rapidly to all parts of the body, and coma and death follow in a few hours.

The "**Furious Form**" form of rabies refers to animals in which **unprovoked aggression** (the acute neural excitative phase) is pronounced. (i) The animal becomes irrational and viciously aggressive. This form is the classical "mad-dog syndrome". (ii) The animal goes into rages (violent anger), biting and slashing at any moving object or even inanimate objects, such as sticks. (iii) The furious champing of the jaws (i.e., chewing noisily) is accompanied by excessive salivation. (iv) The saliva flows from the mouth or is churned into foam, which may adhere to the lips and face. (v) A basic change in **temperament** (behavior) occurs. Animals which normally avoid humans go into the open and attack humans. The rabid dog, fox, or wolf tends particularly to attack a moving person or animal. (vi) As the disease progresses, muscular incoordiation and seizures are common. (vii) Paralysis may follow both in the "furious" or "dumb" stage of the disease. Death occurs within 10 days of the first symptoms and results from progressive paralysis.

Furious Form – all changes described above can be divided into two stages:

a) **Stage of Melancholy*:*** Change in behavior, unusual violence and frenzy behavior, bite inanimate objects, alert condition, eat non-edible object e.g. stone, bone, grass pupils get dilated, altered facial expression.

b) **Stage of Excitement*:*** Becomes aggressive due to excitability & irritability, hide in dark place due to photophobia, hydrophobia, champing of jaw & dribbling of saliva, lick their Genital & show sign of Heat. At the end, the lower jaw will hang, head will drop down, ascending paralysis, coma & death.

Rabies in **Cattle** follows the same general pattern. Those with the furious form are dangerous, attacking and pursuing man and other animals. Lactation stops abruptly in dairy cattle. A most typical clinical sign in cattle is a characteristic **Bellowing** (making a deep loud cry). This may continue intermittently until shortly before death.

Horses and mules show extreme agitation evidenced by rolling as with colic. They may bite or strike viciously, and because of size and strength, become unmanageable in a few hours.

Lesions

There are no specific Gross Lesions. The lesions of rabies are microscopic, limited to the central nervous system, and extremely variable in extent. The typical histological signs are multifocal, polioencephalo-myelitis and craniospinal ganglionitis with mononuclear perivascular infiltrates, diffuse glial proliferation, regressive changes in neuronal cells and glial nodules. Intra-cytoplasmic inclusions k.a. Negri bodies can be seen in few (not all) but is certainly pathognomonic.

Sometimes, there is **diffuse encephalitis** characterized by perivascular cuffing, neuronophagic nodules and destruction of neurons throughout the brain. Histopathologic evidence of rabies encephalomyelitis (inflammation) in brain tissue and meninges includes the following:

1. Mononuclear infiltration
2. Perivascular cuffing of lymphocytes or polymorphonuclear cells
3. Lymphocytic foci
4. Babes nodules consisting of glial cells
5. Negri bodies

These changes are particularly prominent in the **brain stem, hippocampus and the gasserian ganglia**. (**Gasserian ganglion** lies on the sensory root of the trigeminal nerve, and from it originate the three branches - ophthalmic, maxillary, mandibular). Lesions in the gasserian ganglia are specific, develop earlier and are more constant than elsewhere.

The main lesion in the gasserian ganglia consists of the collections of proliferating glial cells encroaching on the neurons and replacing them. These collections of proliferating glial cells are known as "**Babes' nodules" / Microglial Nodules**.

In 1903, spherical cytoplasmic inclusion bodies with specific Tinctorial (Staining) characteristics were described by Negri (a scientist), in the neurons of dogs and cats infected with rabies virus. These inclusion bodies have been called "**Negri bodies**", and are accepted as specific indications of infection with rabies virus.

Negri bodies are always **Intracytoplasmic**, well-defined, electro-dense masses, having distinct limiting membrane, and may be encircled by a narrow, clear halo. (i) In **the Dog,** they are found mostly in the **Hippocampus**, (ii) but **in Cattle** they are more numerous **in the Purkinje cells of the Cerebellum**. A granular, slightly basophilic internal structure can be seen in preparations with Mann's stain.

The haematoxylin and eosin method does not differentiate Negri bodies well. In impression smears, **Seller's Stain** is effective and the inclusion body is bright red or magenta against the pale blue background of the neuronal cytoplasm. **Mann's stains** are also useful.

When the virus centrifugally invades the salivary gland, degenerative changes leading to necrosis may be seen in the acinar epithelium, mainly affecting mucogenic cells of the mandibular salivary gland. Virus can readily be demonstrated within these cells by fluorescent antibody techniques (FAT), and electron microscopy. A moderate infiltration of lymphocytes and plasma cells accompanies the degenerative changes.

Groups of tiny spherical bodies without a limiting membrane are seen in the cytoplasm of neurons in non-rabid animals. At one time they were thought to be associated with rabies, they were called "**Lyssa bodies**". It is now clear that they are not specific for rabies.

Diagnosis

The diagnosis of rabies can be based on the symptoms if they are typical, but should be confirmed by laboratory examination. Dogs or other species, suspected of rabies because of abnormal behaviour, should be kept in isolation cum observation for

atleast 10 days. If the animal is rabid, it will die and the diagnosis can then be confirmed by laboratory examination. The laboratory examination includes the following four tests. It is essential that at least two of them be used on all specimens.

1. A **Fluorescent Antibody Test** (FAT) and **Immunofluorescence Test** on impression smears from the brain is test of choice (**OIE Gold Standard Test that detects rabies virus antigen in brain samples**), preferably hippocampus, medulla oblongata, cerebellum or gasserian ganglion. The test can be completed in about 2 hours and is highly accurate. The reliability is over 99%. For sampling Skull must be opened; however if the skull cannot be opened there are two alternative routes for collected brain samples: the **occipital foramen** route and **retro-orbital route**.
2. A histological staining for search of **Negri bodies** in tissue sections (**Seller's / Mann's Staining**). However, there is limitations, as 30% of infected animals may not contain demonstrable Negri bodies.
3. **Habel's Mice Inoculation Test:** those specimens which are negative on FAT, and had contacts with humans, are inoculated into experimental newborn mice. Newborn mice less than 3 days of age are most sensitive. Following intracerebral inoculation, newborn mice usually die within 14 days, but should be examined daily for at least 4 weeks before the test is considered negative.
4. Recently, a newer **Peroxidase - Antiperoxidase Staining Technique** has been used. It can be performed on paraffin-embedded tissue, and has largely replaced reliance on the presence of Negri bodies, or mouse inoculation.

Serological Testing is rarely useful for ante-mortem diagnosis because of late or failing seroconversion and the high mortality rate of host species, but is very useful for assessing seroconversion following vaccination and for epidemiological studies.

Similarly, **Molecular Techniques** like reverse transcription polymerase chain reaction (RT-PCR) are also useful but are not recommended currently for routine post-mortem diagnosis of rabies, esp. if brain tissue is available, when the FAT should be used.

Differential Diagnosis from following diseases must be considered:

(i) Canine Hepatitis, (ii) Canine Distemper (for encephalitis), and (iii) Toxoplasmosis

*Pseudorabies (Mad Itch) – is caused by Herpes Virus – just for INFO

RABIES Virus	
Street Virus	**Fixed virus**
Made in Nature	made in Laboratory
Naturally virulent / infective	Attenuated
Incubation Period – is variable and Long	Incubation Period – is Fixed and Short
Negri bodies- Present	Negri bodies- Not usually present
Salivary Glands: Affected	Salivary Glands: Not Affected

Differentiate	
Rabies (Hydrophobia, Rage)	**PseudoRabies** (Mad itch; Aujesky's Disease)
Caused by Lyssa Virus (Rhabdo)	Caused by Herpes Virus
Incubation Period: Long	Incubation Period: very short-24 to 48 hrs
Course upto 10 days	Course is very rapid; death in 2 days
Affects Cerebrum & Cerebellum in CNS	Affects *Medulla oblongata* (bulbar) in CNS
Abnormal behavior: Dull, Aggressive; vocalisation	Abnormal Aggression/Dull behavior: not seen
No Itching	Considerable Itching
Paralysis of Larynx is NOT very often	Paralysis of Larynx is VERY Often
Consciousness may be Lost	Consciousness never Lost
Virus spread: Centrifugal	Virus spread: Centripetal (towards centre)
Inclusion Body: Intracytoplasmic	Inclusion Body: IntraNuclear