RABIES IN ANIMALS: PART II
DIAGNOSIS AND CONTROL MEASURES

*Lahane Sunil R., Bhuktar V.M., Ganvir P.T., and Rautmare S.S.
Western Regional Disease Diagnostic Laboratory
Disease Investigation Section
Aundh, Pune – 411 007.

Rabies (Latin: rabies, “madness, rage, fury”)

Rabies is an acute and fatal viral encephalitis caused by a single stranded RNA virus belonging to the genus Lyssavirus of the family Rhabdoviridae. It is one of the oldest diseases known to mankind that continues to kill thousands of people every year in spite of the availability of effective vaccines and sera to prevent it. It is primarily a zoonotic disease transmitted to man by the bites from infected animals. Once symptoms of the disease develop, rabies is fatal to both animals and humans.

Rabies is transmitted by the bite of an animal with rabies (a rabid animal) or through close contact with saliva from infected animals (i.e. bites, scratches, licks on broken skin and mucous membranes). Rabies is found throughout the world.

Rapid and accurate laboratory diagnosis of rabies in humans and other animals are essential for timely administration of postexposure prophylaxis. In addition, laboratory identification of positive rabies cases may aid in defining current epidemiologic patterns of disease and provide appropriate information for the development of rabies control programs.

Rabies is a vaccine-preventable disease, and it is still a significant public health problem in many countries of Asia and Africa, even though safe, effective vaccines for both human and veterinary use exist.

1. Livestock Development Officer (Pathology)
2. Deputy Commissioner of Animal Husbandry (Poultry)
3. Joint Commissioner of Animal Husbandry
4. Deputy Commissioner of Animal Husbandry (Virology & FMD)
Diagnosis of rabies

Brain tissue is the preferred specimen for postmortem diagnosis in both humans and animals.

Diagnosis in Animals can be done by the following ways:

1. **History**: of bites with rabid animal.

2. **Clinical signs**: Clinical diagnosis is difficult, especially in areas where rabies is uncommon and should not be relied on when making public health decisions. In the early stages, rabies can easily be confused with other diseases or with normal aggressive tendencies. Therefore, when rabies is suspected and definitive diagnosis is required, laboratory confirmation is indicated. Suspect animals should be euthanized and the head removed for laboratory shipment.

3. **Laboratory diagnosis**: Rabies testing should be done by a qualified laboratory, designated by the local or state health department in accordance with established national standardized protocols for rabies testing. The following tests are used for laboratory diagnosis:

   1. **Immunofluorescence** microscopy on fresh brain tissue, which allows direct visual observation of a specific antigen-antibody reaction, is the test of choice. When properly used, it can establish a highly specific diagnosis within a few hours.

   2. **The mouse inoculation test** or **tissue culture techniques** using mouse neuroblastoma cells may be used for indeterminate fluorescent antibody results.

   3. **ELISA** and **PCR techniques** can be applied for epidemiological surveys in laboratories with strict quality control procedures.

   4. **Virus isolation and Antibody titration**.

   5. **Histopathology**.

   6. **Immunohistochemistry (ICH)**.

   7. **Electron microscopy (EM)**: is a useful tools for studying the virus structure, histopathology, molecular typing, and virulence of rabies viruses.

**LABORATORY TESTS FOR RABIES**

1. **Direct fluorescent antibody test (dFA)**

   The standard test for rabies testing is dFA. This test has been thoroughly evaluated for more than 40 years, and is recognized as the most rapid and
reliable of all the tests available for routine use. Impressions (or smears) of tissue samples from brainstem, thalamus, cerebellum, and the hippocampus (Ammon’s horns) are recommended for increased sensitivity of the test. Brain tissues examined must include medulla oblongata and cerebellum (and should be preserved by refrigeration with wet ice or cold packs).

The dFA test is based on the observation that animals infected by rabies virus have rabies virus proteins (antigen) present in their tissues. Because rabies is present in nervous tissue (and not blood like many other viruses), the ideal tissue to test for rabies antigen is brain. The most important part of a dFA test is fluorescently-labelled anti-rabies antibody. When labelled antibody is incubated with rabies-suspect brain tissue, it will bind to rabies antigen. Unbound antibody can be washed away and areas where antigen is present can be visualized as fluorescent-apple-green areas using a fluorescence microscope. If rabies virus is absent there will be no staining.

Antigen detection by dFA: The rabies antibody used for the dFA test is primarily directed against the nucleoprotein (antigen) of the virus. Rabies virus replicates in the cytoplasm of cells, and infected cells may contain large round or oval inclusions containing collections of nucleoprotein (N) or smaller collections of antigen that appear as dust-like fluorescent particles if stained by the dFA procedure. (See Fig.1).

2. General histopathology

Histologic examination of biopsy or autopsy tissues is occasionally useful in diagnosing unsuspected cases of rabies that have not been tested by routine methods. When brain tissue from rabies virus-infected animals are stained with a histologic stain, such as hematoxylin and eosin, evidence of encephalomyelitis may be recognized by a trained microscopist. This method is nonspecific and not considered diagnostic for rabies.
Before current diagnostic methods were available, rabies diagnosis was made using this method and the clinical case history. In fact, most of the significant histopathologic features (changes in tissue caused by disease) of rabies infection were described in the last quarter of the 19th century. After Louis Pasteur’s successful experiments with rabies vaccination, scientists were motivated to identify the pathologic lesions of rabies virus.

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 (Fig. 2).
3. Lymphocytic foci
4. Babes nodules consisting of glial cells (Fig. 3).
5. Negri bodies

Perivascular cuffing or inflammation around a blood vessel. Perivascular inflammatory cell infiltrates in hematoxylin & eosin stained brain tissue. (See Fig. 2).

Fig. 2. Perivascular cuffing

Negri bodies

Negri bodies are as round or oval inclusions within the cytoplasm of nerve cells of animals infected with rabies. Negri bodies may vary in size from 0.25 to 27 μm. They are found most frequently in the pyramidal cells of Ammon’s horn, and the Purkinje cells of the cerebellum. They are also found in the cells of the medulla and various other ganglia. Negri bodies can also be found in the neurons of the salivary glands, tongue, or other organs. Staining with Mann’s, giemsa, or Sellers stains can permit differentiation of rabies inclusions from other intracellular inclusions. With these stains, Negri bodies appear
magenta in color and have small (0.2 μm to 0.5 μm), dark-blue interior basophilic granules. (Fig. 4).

The presence of Negri bodies is variable. Histologic staining for Negri bodies is neither as sensitive nor as specific as other tests. Some experimentally-infected cases of rabies display Negri bodies in brain tissue; others do not. Histologic examination of tissues from clinically rabid animals show Negri bodies in about 50% of the samples; in contrast, the dFA test shows rabies antigen in nearly 100% of the samples. In other cases, non-rabid tissues have shown inclusions indistinguishable from Negri bodies. Because of these problems, the presence of Negri bodies should not be considered diagnostic for rabies.

H & E-strained tissues

Fig. 4. Neuron without Negri bodies

Negri body in infected neuron

Sellers stained brain tissue

Fig. 5. Negri body in Sellers stained brain tissue.
Note the basophilic (dark blue granules in the inclusion)

3. Immunohistochemistry (IHC)

IHC methods for rabies detection provide sensitive and specific means to detect rabies in formalin-fixed tissues. These methods are more sensitive than histologic staining methods, such as H&E and Sellers stains. Like the dFA test, these procedures use specific antibodies to detect rabies virus
inclusions. The techniques use enzyme-labeling systems that increase sensitivity. In addition, monoclonal antibodies may be used to detect rabies virus variants.

**Histological section of brain from a rabid animal**

Fig. 6. The slide shows a rabies virus-infected neuronal cell with intracytoplasmic inclusions. The red stain indicates areas of rabies viral antigen by using IHC or avidin-biotin complex (ABC) technique.

4. **Electron microscopy / Ultrastructure**

The ultrastructure of viruses can be examined by electron microscopy. Using this method, the structural components of viruses and their inclusions can be observed in detail. Rabies virus is in the family of Rhabdoviruses. When viewed with an electron microscope Rhabdoviruses are seen as bullet-shaped particles. (Fig. 7).

**Negatively stained Rhabdovirus as seen through an electron microscope**

Fig. 7. a) Notice the bullet shape of the virus. b) See the “beehive” like striations of the RNP. c) Notice the glycoprotein spikes in the outer membrane bilayer.

5. **Amplification methods**

Samples containing small amounts of rabies virus may be difficult to confirm as rabies-positive by routine methods. Virus isolation in cell cultures increases the virus concentration because the virus replicates in cell cultures. Mouse neuroblastoma cells (MNA) and baby hamster kidney (BHK) cells provide an excellent environment for amplification of rabies virus without the use of animals.

Another method for amplifying the nucleic acid portion of rabies virus uses biochemical methods. With this procedure, rabies virus RNA can be enzymatically amplified as DNA copies. Rabies RNA can be copied into a DNA molecule using reverse transcriptase (RT). The DNA copy of rabies can then be amplified using polymerase chain reaction (PCR). This technique can confirm dFA results and can detect rabies virus in saliva and skin biopsy samples.
Biosafety considerations

Rabies has the highest case-fatality rate of any currently recognized infectious disease. Safety is of paramount importance when working with lyssaviruses. In general, biosafety level 2 safety practices are adequate for routine laboratory activities such as diagnosis and animal handling. Besides basic facility design, precautions should also include personal protection equipment (e.g. clothing) and pre-exposure vaccination. Certain situations may entail consideration of a biosafety level 3 classification, including production of large quantities of concentrated virus, conducting procedures that may generate aerosols and when working with lyssaviruses for which the effectiveness of current prophylaxis is not known. All national safety guidelines for working with infectious agents should be followed.

Transport of specimens

Specimens for rabies diagnosis should be shipped according to the national and international regulations to avoid exposure hazards.

Diagnostic specimens should either be refrigerated or shipped at room temperature in 50% glycerine-saline solution.

For short distance, keep specimen in tight container and send
immediately on ice. For long distance, dry ice may be necessary or in 50% GPBS. Half of the brain should be sani in 10% formalin.

**Source of specimens for diagnosis and storage conditions**

Rabies diagnosis can be performed on fresh specimens from several different tissue sources or on appropriate specimens stored at proper temperatures, preferably refrigerated. The choice of specimens depends on the test to be performed and the stage of the disease in humans.

Formalin fixation of brain tissues is not recommended. If specimens are nevertheless received in formalin, the duration of fixation should be less than 7 days. The specimens should be transferred rapidly to absolute ethanol for subsequent molecular diagnosis.

**Sampling for intra vitam diagnosis**

Secretions and biological fluids (saliva, spinal fluid, tears, etc.) and tissues can be used to diagnose rabies during life (intra vitam). They should be stored at -20 °C or below. Serum should be collected from blood samples prior to freezing and stored at -20 °C or below.

**Sampling for postmortem diagnosis**

Brain tissue is the preferred specimen for postmortem diagnosis in both humans and animals. In cases where brain tissue is not available, other tissues may be of diagnostic value. In field studies or when an autopsy cannot be performed, techniques of collecting brain-tissue samples via trans-orbital or trans-foramen magnum route can be used. The use of glycerine preservation (temperature: +4 °C or -20 °C) or dried smears of brain tissue on filter paper (temperature: +30 °C) also enables safe transportation of infected material.

**Treatment**

Rabies is a fatal disease. There is no specific treatment for the disease except management.

If there is an animal bite, there are a few simple steps you can take:

- Wash the wound with lots of soap and running water.
- Followed by the application of ethanol, tincture or aqueous solution of iodine.
- Give tetanus vaccination.
- Give antibiotics if required.
If possible and without causing further injury, try to identify or capture the biting animal.

If the biting animal is a dog, cat or ferret, it can be observed for 10 days after the bite. If it is not ill after that time, you were not exposed to rabies.

You can avoid being exposed to rabies by doing the following.

- Don’t attempt to pet animals unknown to you
- Don’t approach animals that are sleeping, injured, eating or caring for young
- Avoid contact with wild animals; enjoy them from a distance
- Exclude wildlife access to your house, garage, etc.
- Don’t leave pet food out where it will attract wildlife
- Keep garbage containers closed and secure
- HAVE YOUR PETS VACCINATED AGAINST RABIES - a vaccinated pet is a barrier between you and rabid wildlife.

Not to Do:

- Do not apply prickle concentrate, kerosene oil, petrol, mobile oil, etc. on the wound.
- Do not go for spiritual/magical/tantrik way of curing. These are of no use and lack scientific credibility.

Prognosis

Exposure to a rabid animal does not always result in rabies. If preventive treatment is obtained promptly following a rabies exposure, most cases of rabies will be prevented. Untreated cases will invariably result in death.

Control

Comprehensive guidelines for control in dogs have been prepared by the World Health Organization and include the following.

1) Notification of suspected cases, and euthanasia of dogs with clinical signs and dogs bitten by a suspected rabid animal;

2) Reduction of contact rates between susceptible dogs by leash laws, dog movement control, and quarantine;
3) Mass immunization of dogs by campaigns and by continuing vaccination of young dogs;

4) Stray dog control and euthanasia of unvaccinated dogs with low levels of dependency on, or restriction by, humans; and

5) Dog registration.

To minimize Rabies risk

(a) Exposure to rabies may be minimized by removing all stray dogs and cats as well as having all pets vaccinated.

(b) Wild animals should not be kept as pets.

(c) Do not allow bats to live in your house attic or chimney. Remember: bats may carry rabies.

(d) If you hunt, use gloves while skinning animals in particular while handling nervous tissue or organs (spine and brain for example).

(e) Avoid picking up dead or abandoned animals, and do not capture or eat animals that do not look or act normal.

Control in dogs

Effective control of rabies in dogs requires the immunization of a large proportion of the dog population over a period of several years to reduce the contact rate between rabid and susceptible dogs to a level too low to sustain rabies transmission within the population.

Parenteral vaccination programs in developed countries have been effective in preventing rabies in dogs. This has resulted in a marked reduction in the incidence of human rabies and post exposure rabies treatments. Parenteral vaccination of pet dogs is not effective for rabies control in countries with large numbers of stray and ownerless dogs as an insufficient number of the total dog population is vaccinated.

In India an attempt to control rabies was made through programs to exterminate the stray dog population. This method proved ineffective. The stray dog population is so large that new packs of dogs quickly moved into areas where dogs had previously been eliminated.

An integrated approach to rabies control

To effectively control rabies in countries with large numbers of stray dogs the following is suggested:
The promotion of responsible pet ownership to dog owners:

1. Parenteral rabies vaccination of owned dogs.
2. Sterilization of pet dogs.
3. Unwanted dogs should not be abandoned.

Animal birth control - attempts should be made to sterilize the stray dog population or other methods of birth control should be investigated.

Suitable infrastructure for garbage disposal - to prevent the accumulation of waste in and around residential areas. This attracts stray and ownerless dog packs to these areas.

Vaccination - Sufficient and affordable cell culture vaccine should be available for post exposure treatment.

Mass oral vaccination of the stray dog population.

Management of stray dog population

India has approximately 25 million dogs, with an estimated dog:man ratio of 1:36. The dogs fall into 4 broad categories: pets (restricted and supervised); family dogs (partially restricted, wholly dependent); community dogs (unrestricted, partially dependent); and feral dogs (unrestricted, independent). Most dogs in India, perhaps 80%, would fall into the last 3 categories.

Until 1998 the population of stray dogs in India was kept under check by civic authorities, by impounding and euthanizing unclaimed dogs. Because of pressure from animal welfare activists, this approach was replaced by a policy of animal birth control, also referred to as the ABC Programme. In this program, stray dogs are impounded, surgically sterilized and released back into
the area from where they were picked up. The success of this program hinges on the sterilization of 70% of the strays in a given geographic area within 6 months, before the next reproductive cycle begins, otherwise the entire effort is negated. This target is difficult to achieve, given the large number of strays and the limited resources. Hence the success of the animal birth control program in controlling the stray dog population is a subject of dispute and doubt.

**Vaccination**

Rabies Veterinary Vaccine is used in animals of all species either for post-exposure treatment or pre-exposure prophylactic immunization against rabies by subcutaneous route.

1. **Post-exposure immunization** :

   i. Non-immunised animals or immunized previously but immunization period is passed away or incomplete previous vaccination – 5 injections one each on day 0, 3, 7, 14 and 28.

   ii. Subject already immunized, and within the immunization period of previous immunization - 2 injections one each on day 0 and 3.

2. **Pre-exposure immunization** :

   i. Primary vaccination - 3 injections one each on day 0, 7 and 21/28.

   ii. Booster injection – one year later.

**References**


Internet Websites.
