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RABIES

- **Rabies is a fatal infection of the central nervous system transmitted by a virus and acquired through the bite of a rabid animal.**
- **The rabies virus still exists in nearly all parts of the world, and rabies is a serious public health concern, both in developed and developing countries.**
- **Over the course of the 20th century, the number of human deaths due to rabies in the U.S. declined from 100 or more annually to an average of 1-2 per year. Still, about 20,000 Americans receive post-exposure rabies treatment each year.**
- **The World Health Organization estimates that approximately 50,000 people die each year from rabies worldwide, more than half of these on the Indian subcontinent. Millions more worldwide are treated each year after being exposed to rabid animals.**
- **Rabies has the broadest host range of any virus. All mammals, including humans, are susceptible to rabies infection.**
- **More than 7,000 cases of animal rabies are reported every year in the U.S.**
- **The primary vector and reservoir of rabies worldwide is the dog. In the U.S., rabies is found most often in raccoons, skunks and bats.**
- **Rabies is the only human disease that can be treated by post-exposure vaccination.**
- **Louis Pasteur and his research team developed the first successful rabies vaccine in 1885.**
- **Vaccines and anti-sera are currently the only means of preventing rabies infection in human and veterinary medicine.**
- **Post-exposure treatment of rabies, primarily in developing countries of the subtropical and tropical regions, is extremely costly.**

Source: Centers for Disease Control

Introduction

Rabies is an infectious and contagious **zoonotic disease** caused by a virus. The rabies virus attacks the central nervous system and travels to the brain, where it ultimately causes the death of the infected person or animal. All mammals are susceptible to rabies infection, including humans.

Rabies is an ancient disease, dating back to the earliest recorded history. The origins of the word "rabies" have been traced back to the Sanskrit, meaning "to do violence." The first recorded description of the disease dates from the 23rd century B.C. in the Eshuma Code of Babylon, which recognized that the bite of a rabid dog could cause death in humans and described

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NOTE:

Words contained in the glossary (pages 19-22) are highlighted in **bold** .

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policies for animal control. The disease was also described in ancient Egyptian writings and later by Democritus (500 B.C.) and Aristotle (322 B.C.) in ancient Greece. Celsus identified saliva as the source of transmission in 100 years A.D. It was the Italian scholar Girolamo Fracastoro who in 1584 first described in detail the clinical disease in humans in *The Incurable Wound*. The first rabies cases in the New World were recorded in dogs in Virginia in 1753.

The Rabies Virus

Rabies is caused by a virus called a ***lyssavirus***, a member of a large family of viruses called ***rhabdoviruses***. The virus particles have a bullet-shaped structure with **RNA** as its genome and five proteins. The glycoprotein G, found within the phospholipid bilayer on the outer surface of the virus, is the rabies antigen responsible for inducing **viral neutralizing antibodies** (VNA) and for stimulation of other immune cells. Rabies VNAs directed against G protein appear to be an important component in the immune response to rabies. The nucleocapsid N protein, found in the inner core of the virus, is closely associated with the viral RNA. Scientists are able to tell which animal species or geographic region a particular rabies virus came from by examining the protein patterns on the outer coat of the virus.

Rabies is a bullet-shaped virus.

The rabies virus lives in the nerve cells and glands, such as the salivary glands, of its carriers. Like other viruses, it uses infected cells of its animal host, turning them into miniature factories dedicated to producing more viruses according to the instructions provided in the strand of RNA. The new viruses burst from their host cells and can spread to other cells or be transmitted to new animal hosts.

Pathogenicity and Clinical Progression of Rabies

The primary mode of rabies transmission is through the bite of an infected animal, and to a lesser extent, scratching and licking can also transmit the disease. There are also a few documented cases in which transmission has occurred via viral aerosols and cornea transplants. Transmission via a bite will depend on the presence of virus in the saliva at the time the wound is inflicted.

The virus may remain near the entry site for some time until it crosses over into the nerves. Some evidence exists for slow replication of the virus in muscle cells during this latent period.[1]

For domestic animals, clinical signs of rabies usually develop within two weeks to three months after a bite from a rabid animal. The **incubation period** in wildlife, however, is highly variable and has been known to extend as long as a year. In humans, a period of two to eight weeks generally elapses before the onset of symptoms, but incubation periods of over a year have been reported.[2] The length of the incubation stage, during which the person or animal cannot transmit the virus, is dependent on a variety of factors, including the location and extent of the bite wound. Generally, the closer the wound is to the brain, the shorter the incubation period.

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Following the incubation period, transport of the virus occurs along the nerves, from the site of virus entry to the spinal cord and into the brain, which is the preferred site of virus replication. The virus has been reported to move toward the brain at a rate of 100-400 mm per day.[3] Portions of the rabies virus glycoprotein are **homologous** to neurotoxins and the virus attaches to neural axons through **receptors** used by them. The virus then enters the axon and travels to the nucleus of the neuron. It replicates in the neurons and passes to other neurons through the fiber connections.

By travelling through the nervous system, the rabies virus bypasses the host's immune system, which normally operates in the bloodstream and other body tissues. Subsequent to reaching the brain and replicating fully, the virus is transported through the nerves to the peripheral organs. The virus also passes down axons to the skin and to the salivary glands, where it is released into the saliva. Most of the viral damage to the brain is in the hypothalamus, but it is interference with cardiorespiratory control that ultimately results in death.[4]

There are two types of clinical rabies -- often known as **dumb** (or paralytic) **rabies** and **furious** (or classic) **rabies**. About 80% of human rabies cases have the classic form of the disease and 20% have a paralytic form. Both forms can cause abnormal behavior. Regardless of the clinical presentation, once symptoms manifest themselves, rabies is almost invariably fatal.

Immediately prior to death, animals with furious rabies will appear to be "mad" -- travelling great distances, frothing at the mouth and hydrophobia (symptoms caused by drooling due to difficulty in swallowing), and biting aggressively, thereby transferring the virus to other animals. It is this propensity to roam over great distances that rabies travels to areas where it may not have previously existed. Animals with furious rabies may show extreme excitement and attack stationary things or animals. Bouts of furious rabies usually alternate with periods of depression. When the roving period is over, the animal may come home to die following a series of spasms and lapses into a paralytic state.

In dumb, or paralytic, rabies, there is no "mad" period. Paralysis, usually of the lower jaw, and a drooping head, are the first recognizable signs of the disease. The paralysis soon spreads to limbs and vital organs and death quickly follows. Animals with dumb rabies may become depressed and retreat to isolated places. Some may appear friendly, having no fear of humans.

Rabies Epidemiology

The **epidemiology** of human rabies is a reflection of the **epizootiology** of the disease in animals. From the viewpoint of public health, dog and other canid species are the most important vector for humans, being responsible for most infections throughout Asia, Africa, and Latin America. Physicians in the U.S. today, how-

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ever, are unlikely to see a case of human rabies. Vaccination and animal control programs since the 1960s have effectively eliminated domestic dogs as a reservoir for rabies.[4] Similar vaccination programs for cats in the past 20 years have been similarly successful.

The epidemiology of rabies in the U.S., therefore, has changed substantially during the last half century. With the retreat of canine rabies from the U.S. and the rest of the developed world, the source of the disease has shifted to wildlife in these regions. In the United States, rabies is **endemic**, and at times **epidemic**, in raccoons, foxes, skunks and bats. These species account for most animals infected with rabies virus. Only rarely has rabies been found in rabbits, squirrels, rats and opossums. In terms of risk to humans, bats are the most likely rabies threat to transmit rabies to humans in the U.S., in particular the silver-haired bat and the eastern pipistrelle bat . Since 1980, more than half of the human rabies cases in the U.S. have been associated with bats.

Farm animals, particularly cattle, are susceptible to rabies through contact with infected wildlife. Captive animals such as zoo animals can also become infected through exposure to both infected wild and domestic animals. And while rabies in companion animals has decreased over 85 percent in the last 40 years, numerous cases of rabies among dogs and cats are still reported each year.

Typically, once rabies enters an animal population, it does not disappear. The disease often follows a four-year cycle in which it subsides, then recurs. While infected animals die and reduce the population, within about four years the population has increased enough so that survivors can spread the disease again.

Rabies occurs in wildlife at all times of the year. In the U.S., it usually peaks in the spring or winter, coinciding with the breeding season when wild animals have the most exposure to each other. Human exposure is most common in summer when people spend more time outdoors.

One rabies epidemic or **epizootic**, as it is called in animal populations, began in 1977 in the central Atlantic states of Maryland, Virginia and the District of Columbia, the result of raccoons imported into Virginia from the southeastern U.S. In 1991, a serious rabies outbreak ocurred in the the northeastern U.S., where the disease was carried to Pennsylvania, New Jersey, Maryland, Delaware, New York, Connecticut and other states by raccoons in the southern states and by red foxes in Canada.

The major foci of rabies in the world today are the Indian subcontinent, Southeast Asia, and most of Africa. Data from India suggest that there are 30,000 cases each year in that country, and one estimate of the death rate associated with rabies is 35.5 deaths per 1 million people. Worldwide, the World Health Organization estimates that there are 50,000 cases of fatal rabies each year. On a list of worldwide causes of mortality, rabies ranks ahead of yellow fever, polio, and meningococcal meningitis.

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Diagnosing Rabies

There is no unequivocal ***intra vitam*** test to confirm whether rabies virus has infected an animal. Tests for **viral neutralizing antibodies** in serum and cerebrospinal fluid, viral antigen in corneal epithelium, and other tests can all be negative in an animal later diagnosed as rabid by the presence of virus in brain tissue. In most cases, reliance is not placed on a single test, and the modern practice is to confirm the fluorescence antibody test (*vide infra*) by means of inoculation of neonatal mice. In many parts of the world, rabies in people and animals is still diagnosed on the basis of clinical signs and symptoms, at which time it is too late to intervene with treatment.

In the distant past, a person or animal that was bitten, the suspected rabid animal was kept in isolation and watched carefully. Due to the variability of rabies incubation periods, however, especially in wild animals, this method is unreliable and time-consuming. More recently, scientists were able to determine within just 30 minutes if an animal was rabid or not by examining a sample of its brain for **Negri bodies**. These are clumps of virus material containing the rabies RNA that accumulate in the cells when the virus reproduces. These clusters become large enough to be seen under a microscope when they are stained with suitable dyes. Negri bodies can be missed in 20 percent or more of rabies infections, however, so false negatives make it necessary to confirm the results with other tests.

The routine test for postmortem diagnosis of rabies in animals and humans is the **fluorescent antibody** (FA) **test**, developed in 1958. Antibodies against rabies virus, isolated from the blood of animals immunized against the disease, are chemically joined with a dye (fluorescein isothiocyanate), which **fluoresces** in a characteristic color under ultraviolet light. The fluorescent antibodies are mixed with a sample of tissues from the suspected rabid animal. If the rabies virus is present, the antibodies will attach to it, and the fluorescence will be seen under a microscope.

An enzyme-linked immunosorbent assay (**ELISA**) called Rapid Rabies Enzyme Immunodiagnosis (RREID) is based upon detection of a rabies virus antigen in brain tissue. Since the antigen can be visualized with the naked eye, the test can be carried out under field conditions.

Intra vitam diagnosis is possible in humans. Antigen detection is generally sensitive during the first few days following exposure, while viral neutralizing antibodies in cerebrospinal fluid and serum tend to appear after 7-10 days. Viral antigen may be detected by FA in corneal impressions or in skin biopsies. The sensitivity of the FA technique for *intra vitam* diagnosis is limited, however.

Viral neutralizing antibodies (VNAs) in the serum or cerebrospinal fluid of non-vaccinated human patients may be measured either by the mouse serum neutralization test (MNT) or by the rapid fluorescent focus inhibition test (RFFIT), which is more rapid and at least as sensitive as MNT. An ELISA assay using purified rabies glycoprotein G has been used to determine VNA levels in the sera of several species, including humans. The sensitivity of the assay is limited, however.[5]

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Rabies Prevention

Immunization is one of the most important ways of preventing rabies. Widespread vaccination programs have worked in conjunction with animal control programs to produce a drastic decrease in cases of dogs rabies in the U.S. Keeping companion animals protected also removes a potential link between rabid wild animals and human owners of companion animals.

All dogs and cats should be vaccinated against rabies beginning at 3-4 months of age and periodically thereafter. The revaccination interval is 1-3 years, depending on the vaccine used.

Most people have a low risk of rabies exposure, making routine rabies vaccination in people unnecessary . Pre-exposure **prophylaxis** through vaccination is recommended for people who will be exposed to rabies virus in the laboratory or who will have contact with mammals. Included on this list are veterinarians; animal caretakers, such as zoo personnel and laboratory animal specialists; biologists who work with mammals and/or mammalian tissues; mail carriers; and travelers to countries where rabies is a serious threat.

For humans, rabies vaccines may be divided into three types -- nerve tissue vaccines, avian embryo vaccines, and cell culture vaccines. Nerve tissue vaccines are still the most widely used type of rabies prophylaxis worldwide. There are side effects in terms of induction of autoimmune central nervous system disease; in addition, multiple injections are required and the treatment is not always effective. Developed in the 1950s, the Fuenzalida vaccine, prepared from rabies virus grown in **neonatal** mouse brains and **attenuated** using ultraviolet light, was an improvement over prior vaccines in this regard, but it still is known to provoke neurological problems.

In the United States today, there is a choice of three vaccines for human use:

- **Human diploid cell vaccine** (HDCV) is derived from the Pitman-Moore strain of the virus and has been licensed in the U.S. since 1980.[6] The advantages of HDCV and the cell culture vaccines that followed are purity, a high level of **immunogenicity** that permits a rational dosing schedule, and efficacy demonstrated in extensive animal and human clinical trials. It is supplied in both intramuscular (IM) and intradermal (ID) forms.

- Fetal rhesus lung cell culture vaccine (or rabies vaccine adsorbed, RVA), produced by the Michigan State Department of Public Health , is derived from the Kissling strain of rabies virus cultured in fetal rhesus lung diploid cells, and has been licensed in the U.S. since 1988. RVA is formulated for IM administration only.

- The purified chick embryo cell culture vaccine (PCEC), originally developed for dogs, became available for human use in 1997. The PCEC vaccine is prepared from the Flury LEP strain grown in cultures of chicken fibroblasts and formulated for IM administration.[7]

When used as directed, all three vaccines are interchangeable and considered equally safe and effective, but only HDCV is formulated and approved by

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the U.S. Food and Drug Administration for intradermal administration. The disadvantage of these vaccines is their cost of production, particularly for the original HDCV. Subsequently developed vaccines have used less expensive cellular substrates to reduce their price.

Outside of the United States, there are other vaccine choices, including primary hamster kidney cell culture vaccine, monkey Vero cell vaccine, purified duck embryo vaccine, and numerous nerve tissue vaccines. Duck embryo vaccine has not been available in the U.S. since 1981, as allergic reactions to the vaccine were frequently reported.

Until the early 1960s, choice of vaccines for veterinary use was limited. This changed with the introduction of inactivated tissue culture vaccines, following the work of Fenje in Canada.[4] The ERA strain of the virus was adapted to hamster kidney cells, then later adapted by others to pig kidney cells. Such a live vaccine has been used to immunize dogs, cats, cattle, sheep, goats and horses.

Control of rabies in wildlife is difficult to achieve, largely because wild animals are free-ranging. In the first half of the 20th century, public health officials tried to control rabies in wildlife by **culling** the populations of animals that carried the disease. Population reduction was not very successful, however, and other methods were successively developed. Early attempts to immunize wildlife involved capturing, vaccinating and then releasing the animals. Capturing entire populations for vaccination, however, was not realistic.

Self-vaccination of animals is an idea that originated in the 1960s at the the **Centers for Disease Control** (CDC) in Atlanta, GA. During the 1960s and 70s, fifteen different research teams in nine countries worked together to develop and perfect an effective oral vaccine for wildlife. Extensive testing was conducted to ensure that animals would not develop rabies from weakened virus strains.

In 1978, with a rabies epidemic spreading along the eastern shore of Lake Geneva in Switzerland, more than four thousand chicken heads with a vaccine-filled packet placed under the skin were spread by hand over the Rhône river valley. Foxes in the area became immune to rabies and the disease did not spread past the vaccination zone. The plan was later tried in other Swiss valleys with equal success, and the Swiss government began funding wildlife vaccination programs across the country. Later, a more sophisticated method of manufacturing small cubes of liquid vaccine coated with fish meal or other flavoring proved a more practical and desirable form in which to distribute the vaccine to foxes. Switzerland has been effectively rabies-free since 1986. By 1989, five different types of oral rabies vaccines were being used in 12 countries to immunize foxes in Europe.[8]

Oral vaccines with live viruses were not effective with animals other than foxes, however, and foxes were not a major reservoir of rabies in the U.S. In 1984, researchers in Pennsylvania developed a vaccine for raccoons by inserting a gene from the rabies virus into the **vaccinia** virus. Vaccinia is a close relative of the smallpox

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virus, a laboratory strain not found in nature, and was used as a **vector** for vaccines against smallpox. The gene that was inserted carried the hereditary instructions for producing the rabies **glycoprotein** G that projects from the surface of the rabies virus. Rabies glycoprotein is the **antigen** that the body recognizes to identify the rabies virus, and so the body produces antibodies against it. Since the glycoprotein is only a small portion of the entire rabies virus, however, it cannot produce the disease. The combination of vaccinia virus and the rabies glycoprotein gene created a **recombinant** vaccinia virus called VRG (vaccinia rabies glycoprotein). The tiny piece of genetic material used to create it came from a rabies virus that was isolated from a rabid dog in Canada in the late 1960s.[9]

Treating Rabies

A turning point in the history of rabies came in the 1800s with the development by Louis Pasteur of a vaccine with which he was able to immunize dogs. The Pasteur vaccine became the standard treatment for human rabies in 1885, ushering in a new era of immunization. By 1971, the Institut Pasteur in France was supplying large quantities of vaccine for human use prepared from the brains of neonatal mice.

People used to fear rabies treatment almost as much as the disease itself. In the past, large needles had to be injected daily into the tender parts of the abdomen for up to 21 days. Fortunately, rabies treatment today is less drastic.

Rabies can be effectively treated in humans following exposure with prompt wound cleansing, active immunization with a rabies vaccine, and passive immunization by administration of human rabies immune globulin.[10,11]

As soon as possible after being bitten, a person is given a shot of a blood fraction called rabies immunoglobulin containing antibodies against rabies. Half may be injected around the wound site and the other half into the muscles of the buttocks. HRIG helps neutralize rabies viruses right away because it contains antibodies that are specific for the virus. It does not provide lasting protection, however. The injected antibodies gradually break down over a period of weeks. Injecting immunoglobulins to provide immediate but temporary protection from a disease is known as **passive immunization** because it does not involve the body's own immune defenses.

Two forms of rabies immunoglobulin (RIG) exist -- human RIG (HRIG) and equine RIG. However, only human RIG is available in the United States. The currently used HRIGs are purified products and have high **immunogenicity** with essentially no side effects. Because of strict regulation to eliminate extraneous viral contamination, however, human RIG is in short supply.

For long-lasting protection, rabies vaccine is given. Rabies is the only human disease that can be treated by post-exposure vaccination. This form of

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treatment is possible because of the extended incubation period following exposure to the virus. [*See pages 6-7 for a discussion of modern rabies vaccines.*]

About 20,000 Americans are vaccinated each year following rabies exposure.[12] Worldwide, more than half a million people receive rabies treatment each year.[13]

How have animal studies helped in the study of rabies?

Folk Remedies v. Science

Prior to modern medicine based on careful scientific study, many unorthodox ideas were presented as cures for rabies. In ancient times, eating livers of rabid dogs or a paste made of crayfish eyes was thought to be a cure. In the 1600s, a common practice was to submerge patients in a lake or river until they nearly drowned. It was believed that because rabies caused a fear of water, immersion in water would shock the person's system to reverse or expel the disease; many patients are documented to have drowned in the process.

Medicinal preparations in the 17th and 18th centuries often contained such ingredients as asses' milk, children's urine, and the "stones" of a hedgehog. By the mid 1800s, the venom of a viper was thought to counteract the "poison" of rabies. Other folk remedies for rabies included treating the bite wound with ashes of seahorses, with the gallstones of a rare white deer, or with a little burnt hair from the tail of a rabid dog. Still other treatments of this time involved bleeding with leeches, doses of liverwort with black pepper, and "stifling a miserable wretch between two feather beds." [4]

Two early treatments for rabies were somethat effective, thought drastic. Galen, a 2nd century Greek physician, suggested amputating limbs that were bitten. If done early enough, amputation of affected limbs can often prevent the disease from developing. A hundred years earlier, the Roman medical writer Celsus had noted that the saliva of rabid animals transmitted the disease. Celsus prescribed remedies such as bathing in the sea and **cautery**. Cautery was performed by many doctors over the centuries with a red-hot iron, with acids, and even by pouring gunpowder on the wound and igniting it.

Treatments such as those described above persisted over thousands of years all over the world. Because of lack of understanding of the disease, its mode of transmission and lethality, and the drastic nature of most treatments, fear of rabies has been widspread during much of recorded history. As science began to be used more and more to solve societal problems, however, understanding of rabies and the virus transmitting the disease evolved, treatments were developed, and fear of the disease has subsided, particularly in the developed world.

In 1804, Zinke infected healthy dogs with rabies by injecting them with infected saliva from rabid dogs. This demonstrated scientifically for the first time the cause-and-effect that people has suspected for centuries. Zinke wrote a book about the disease, recommending such practical measures as running warm water over the wound for several hours, and cautioned against touching infected saliva with bare hands.

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The work of Zinke and others provided a solid scientific basis for preventing rabies, and officials in Denmark, Norway and Sweden were quick to apply this knowledge to public health policy. By 1826, rabies was successfully eliminated in these countries by enforcing strict laws for dogs.[14]

It had been assumed and accepted for centuries that once it enters the body, the rabies virus permeates the nerves. It was not until 1965, however, that experiments in mice confirmed that the rabies virus in fact moves centrally via the peripheral nerves, reaching the spinal cord in 24 hours and eventually the brain.

Study of Rabies Virus and its Pathogenicity

In the 1880s, Louis Pasteur (*vide infra*) was working with an invisible organism about which he knew little. It was not until the 1960s, nearly a century later, that the size and shape of the rabies virus was described in convincing detail. In 1936, the approximate size of the virus had been estimated by means of filtration experiments. In 1962, electron microscopy was used to find elongated particles in mouse nerve cells infected with the rabies virus. Around the same time, the Pasteur Institute in France reported the presence of bullet-shaped particles in infected animal cells. In 1967, scientists in Philadelphia published a detailed description of the virus grown in cultures of mouse nerve cells and hamster kidney cells. These studies allowed the rabies virus to be classified as a **rhabdovirus**, with spike-like projections on its surface, information that was indispensable in the development of later anti-rabies vaccines.

Immunopathologic studies in mice showed that **viral neutralizing antibodies** (VNAs) are an essential component of the protective response.

With the advent of **monoclonal antibodies** in the 1980s, developed using primarily rodent models, a new tool of **immunology** became available for biomedical researchers studying rabies. All strains of rabies virus were once considered to be closely related. Monoclonal antibody technology has been used to detect antigenetic differences among strains of rabies virus, allowing differentiation of the types and strains of lyssaviruses which may influence the protective capacity of antirabies vaccines. It is now known that the rabies virus in bats, for example, is distinct from terrestrial strains. In addition, the strain found in racoons in the southeastern U.S. is distinct from that found in foxes in the Northeast. These findings are critical to **epidemiologists**, who trace the origins of diseases and epidemics among human and animal populations, as well as to the development of vaccines.

Rabies Diagnosis

In addition to immunization, modern methods of laboratory diagnosos of rabies were developed through research with animals. A current technique of laboratory diagnosis is the **fluorescent antibody** (FA) **test**, in which thin brain smears coated with fluorescein-labelled anti-rabies globulin from a horse are tested for fluorescence under ultraviolet light.

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The diagnosis of rabies is often confirmed through inoculation of mice. Typically, the brains of three to five **neonatal** mice are inoculated with a brain suspension of an animal suspected of carrying the disease. Another three to five mice are inoculated with a salivary gland suspension, and symptoms are observed over a 6- to 14-day period.

Work of Louis Pasteur

By the 1880s, the French chemist Louis Pasteur had already proven that some contagious diseases are caused by "germs." At the time, the nature of viruses was still a mystery. In 1880, Pasteur began to experiment with rabies, believing that it, too, was caused by a germ, in order to isolate the **pathogen** causing the disease. Pasteur's early work with rabies, inoculating rabbits with the saliva of rabid animals, was inconsistent in inducing the disease in experimental animals, and so he made little progress in isolating the virus.

Pasteur then examined the nervous system, as many rabies symptoms -- including paralysis and difficulty in swallowing -- involved the nervous system. When the brains of healthy dogs were injected with a solution made from the pulverized spinal cord of a rabid dog, within two weeks all the dogs developed symptoms of rabies and died within five days. In this way, Pasteur had developed a reliable way to transmit the disease in order to study it.

A turning point in the history of rabies came when Pasteur turned his attention to developing a vaccine for rabies. He had already produced a vaccine for chicken cholera by injecting healthy chickens with cholera germs that were weakened, or **attenuated**. In working with rabbits, he found that the longer a piece of infected spinal cord was exposed to the air, the longer it took for rabies symptoms to develop. After 14 days, the spinal cord mixture produced practically no effect at all.[4]

Pasteur then injected a healthy dog with the 14-day-old spinal cord mixture. The next day, he injected the same dog with a 13-day-old spinal cord solution. Each day, he injected a solution that was one day more potent until, on the last day, he injected a fresh solution that ordinarily would kill the animal within 10 days. Ten days later, the dog was still healthy. The dog remained healthy even after its brain was inoculated with a lethal dose of spinal cord mixture.[4] By June 1885, Pasteur had 50 dogs of various breeds and ages successfully immunized and able to withstand a challenge dose of **virulent** virus.

In the summer of 1885, a 9-year-old boy named Joseph Meister was severely bitten by a rabid dog and taken to Pasteur. Acquiesing to the boy's parents, Pasteur agreed to try the new vaccine treatment. Two weeks later, after receiving the final inoculation, the boy was still healthy. The vaccine was proclaimed a success, and the achievement marked the beginning of the modern era of immunization. Over the following year, 2,500 people who had been bitten by possible rabid animals were given Pasteur's rabies vaccine. By 1935, more than 50,000 people had been treated at the Institut Pasteur, and the Pasteur treatment had become standard practice worldwide .[15]

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Modern Vaccines and Vaccine Testing

Since Pasteur's crucial work, there have been important developments in rabies immunization. The Pasteur treatment for rabies, used until the 1970s, was made from the brain tissue of rabid rabbits. Unfortunately, the technique involved administration of 14 or more vaccine injections given subcutaneously in the abdominal area, making the treatment a painful experience in itself. In addition, the standard Pasteur treatment has side effects and is not effective against bat strains of the virus.

Pasteur's technique was modified in 1908, when Fermi used phenol to attenuate the virus instead of relying on drying infected spinal cords. In 1919, Fermi's method was modified by Semple, who used both phenol and temperature of 37°c to attenuate the virus. Semple-type vaccines have been used throughout the world for both human and veterinary medicine.

Lower-cost vaccines have been developed for use in developing countries. These various vaccines are prepared in the brains of neonatal mice, in hamster kidney cells, in duck or chicken embryos (specially purified to remove allergy-producing bird proteins), and in **Vero cells** (a culture of monkey kidney cells).

In veterinary medicine, the emphasis is placed on prophylaxis rather than post-exposure treatment. Japan is believed to be the first country to use canine vaccination on a large scale in order to control rabies. A Semple-type vaccine of rabbit brain origin was developed in the early 1920s, and its success aroused interest in the U.S., where research on methods to assess the potency of this type of vaccine, and on establishing the duration of immunity in vaccinated dogs, was carried out.

Strict regulations prevent a vaccine from being used in human or veterinary applications until it passes tests in animals to determine both its safety and efficacy. The production of immunological medicines is a biological process and, therefore, is inherently variable. All the production processes have to be strictly controlled to ensure a safe, reliably efficacious and consistent vaccine. Therefore, each batch of vaccine is checked for quality by a panel of tests, most of them requiring the use of animals. Besides analytical tests to guarantee a consistent, validated production process, the safety and potency of products have to be shown. Since the introduction of tissue culture vaccines more than 25 years ago, a minimum potency level has been required. Potency levels were chosen when early studies in animals indicated no significant increase in antibody response above that dose.

In 1961, the **Centers for Disease Control** (CDC) in Atlanta, GA, began to test ways to "self-vaccinate" animals. Early mechanical devices developed to inject wild animals when they tried to eat bait proved hazardous to wildlife. In the late 1970s, however, the CDC looked to incorporation of a vaccine within a bait that animals could eat. A variety of animal species, including raccoons, foxes and mongooses, were used during the development of an oral wildlife vaccine.

In rabies research, "animal research" includes the use of free-ranging wildlife. Vaccinia rabies glycoprotein (VRG, *page 8*) was the first live, recombi-

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nant rabies vaccine for wildlife to be field-tested in the U.S. To ensure the safety and stability of such oral rabies vaccines for wildlife, VRG was tested both in the laboratory and in the field. Many years of research and observation on bait uptake; efficacy; and behavioral studies of the target species following oral vaccination, formed the scientific background for field trials.[16,17,18]

To test the safety and efficacy of the vaccine prior to field testing, sufficient numbers of animals in the target species had to be given the vaccine and challenged with the virus. In addition, since children, adults and other animals may come into contact with oral rabies vaccine intended for wildlife, oral rabies vaccines such as VRG have to be tested first for safety in non-target species. Many studies have shown that VRG vaccine is not pathogenic in over 35 mammalian species, including the majority of rabies reservoir hosts.

In 1990, field testing was conducted on VRG on Parramore Island, an uninhabited, isolated island off the coast of Virginia. The raccoons on the island did develop rabies antibodies and the vaccine was determined to be safe both for the environment and for the other animals on the island. Other field trials were conducted in New Jersey and Pennsylvania to determine the vaccine's ability to halt the spread of rabies in raccoons.

What non-animal methods are used in conjunction with animal models in rabies research?

Non-animal methods have been an important component of the study of rabies, particularly in the past 50 years.

Cloning and sequencing techniques, together with other techniques such as the polymerase chain reaction (PCR), have been successfully applied to the rabies virus and led to characterization of certain regions of the viral genome. PCR has also been used to confirm the results of immunofluorescence tests of tissue from which virus isolation is impossible (formalin-fixed tissue or decomposed brain tissue).[19] One of the greatest uses of PCR, however, has come from epidemiologic studies in which precise identification of a rabies virus variant has provided information about patterns of disease transmission.[20]

Cell culture techniques have been used to study cell receptors that are used by rabies virus to gain entry into cells.[21]

In the past, immunoglobulins -- given to bite victims as part of rabies treatment to stop the rabies virus before it invades the nervous system -- were produced in horses, but these often produced serious allergic reactions. The human immunoglobulin (HRIG) that replaced it is much safer and less expensive, though its supply was originally not sufficient to treat everyone who needed it.

Researchers now use biotechnology to produce better and less expensive immunoglobulins against rabies. Cultures of specialized cells called **hybridomas** are used to manufacture extremely pure specific antibodies. A hybridoma is a combina-

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tion, or "hybrid," of two kinds of cells -- an antibody-producing immune cell and a hardy, fast-growing cancer cell. When challenged by an antigen, each hybridoma cell produces its own, unique antibody against some portion of it. The individual hybridoma cells are multiplied into huge cultures, in each of which all the cells share exactly the same heredity. Such a culture of identical cells is called a **clone**. The single, very specific antibody produced by a hybridoma clone is referred to as a **monoclonal antibody**.

Early rabies vaccines were made from the brain tissue of infected adult animals. These vaccines sometimes caused encephalitis, a brain infection, as well as other less serious side effects. The solution to problems of safety with early rabies vaccines was found in the development of vaccines prepared from virus grown in cell cultures free from neural tissue. The human diploid cell culture vaccine (HDCV) was the first cell culture vaccine; after four years of clinical studies in thousands of human volunteers, the vaccine has been used in humans since 1980. Another of these is the PCEC vaccine using chicken fibroblast cells. The cell culture rabies vaccines are among the safest and most **immunogenic** vaccines. Many studies showed their lack of neurocomplications and high immunogenic property and protective value.

Using the fluorescent antibody (FA) test to diagnose rabies, the virus can be detected in the brain of an injected baby mouse after only four days. A newer version of the FA test uses cultures of mouse nerve cells instead of live mice. The brain sample from the suspected animal is injected into the nerve cells. If rabies virus is present, it will be visible within four days.

The method currently recommended by the World Health Organization for the potency assay of rabies vaccine is the NIH mouse potency test, or **NIH test**. Mice are vaccinated intraperitoneally with several vaccine dilutions. Two weeks later, they are challenged with an intracerebral injection of rabies virus under anaesthesia. Overall, the rabies infection in mice is characterised by a slow onset of disease usually beginning between 4 and 6 days after infection. More than 120 mice are needed for potency tests for each batch of a rabies vaccine.

Several ***in vitro*** methods for replacement of the NIH test are under development. However, none has yet been validated in an international multicenter study.

In the 1980s, titres from the National Institutes of Health given by six laboratories for three vaccines were compared with those given by 14 laboratories using *in vitro* tests, such as the Single Radial Immunodiffusion test (SRID), an assay for detection of the rabies virus glycoprotein. Unfortunately, it was found that the *in vitro* tests do not accurately reflect the protective activity of vaccines. In addition, challenge with a field strain of the virus is difficult to carry out as it requires a huge amount of challenge virus.

Some studies have focused on the use of monoclonal antibody-based **ELISA** systems for quantitative detection of rabies glycoprotein in vaccines for human and veterinary use. These studies may lead to production of convenient ELISA kits as a suitable assay for rabies vaccine potency testing.

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One alternative approach that scientists have developed is the use of **humane endpoints**, a **refinement** alternative. A humane endpoint can be defined as the earliest indicator in an animal of pain, distress or impending death. This can be used to terminate exposure or start therapy, thus allowing the animal to be humanely euthanized or to recover as soon as the scientific objective has been achieved. Ideally, the endpoint should be easy to monitor, be reproducible, and be a valid predictor of death. Furthermore, it should result in the greatest reduction in animal pain and distress, in both intensity and duration. Humane endpoints are identified and described when an experiment is being planned and are incorporated into the experimental protocol.

Since vaccine challenge tests such as the NIH test will continue to be used to estimate the potency of some vaccines for human and veterinary applications, scientists are evaluating and validating the use of humane endpoints as an alternative to severe clinical and lethal endpoints in vaccine potency tests. In the NIH test, scientists are looking at loss in body weight and the appearance of well-established signs of neurological disorder as humane endpoints for terminating rabies vaccine potency experiments without any loss of scientific data.

Virtually 100% of healthy individuals will mount an adequate immune response with appropriate administration of culture rabies vaccines. However, concerns have been raised with respect to the adequacy of long-term protection. Studies have been conducted among veterinary students, Peace Corps volunteers, and others who generally receive prophylactic rabies vaccination, to establishing the length of time viral neutralizing antibodies are present following vaccination.[22]

Epidemiology is the mathematical modelling of disease epidemics. Date obtained through wildlife surveillance are used to assess and refine mathematical models of rabies **epidemics** and **epizootics**. Such models have been developed to explore the population dynamics of infectious disease management in people and wildlife. An epizootic of raccoon rabies in the mid-Atlantic region of the U.S. in the late 1970s developed into one of the largest and most extensive in the history of wildlife rabies. Epidemiologists were able to analyze this, as well as localized outbreaks, over a 20-year period to compare the size, number and periodicity of rabies epizootics among raccoons and develop computer models of raccoon rabies.[23] A recently published mathematical analysis of a rabies epidemic determined that major rivers can slow the spread of rabies by almost a year, resulting in a seven-fold reduction in the transmission rate.[24]

Do we still need to use animals in rabies research?

Biomedical science has evolved tremendously from the days when local wound cauterization was a preferred method for treating people exposed to rabies. After thousands of years of the rabies threat to human and animal populations, with appropriate wound care and vaccination procedures, rabies in people and animals has been a preventable disease for more than a century because of scientific exploration of the disease and the virus causing it.

Cover

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Despite these advances, rabies continues to pose a serious public health problem worldwide. While dogs no longer present a rabies threat in the U.S., 90 percent of rabies cases in people worldwide still originate from bites by rabid dogs. In addition, the incidence of rabies in domestic and farm animals of economic importance impairs the development of animal production and inflicts economic losses in many developing countries.

While controlling rabies is a matter of immunizing wildlife and domestic animals, researchers need to understand better how the virus acts at the molecular level. There are still many aspects of the pathogenicity of rabies that are not entirely understood, e.g., the long incubation period of the virus following exposure. With a better understanding of the action of the rabies virus from a molecular perspective, vaccines and drugs to neutralize the virus can be sought.

New patterns of rabies infection present a problem for both epidemiologists and virolosists. The rabies virus has been isolated from the mammary glands of several mammalian species, demonstrating the possibility of transmission from mother to unborn child. this is an area requiring further validation and study. New wildlife hosts have emerged, and emerging strains of the rabies virus in animal reservoirs have been identified. Rabid bats have emerged as a concern. Every U.S. state except Hawaii and every province in Canada has reported rabid bats. "Cryptic rabies," a condition in which a person contracts rabies despite no evidence of exposure, has become a problem. Scientists need to understand more about how the rabies virus is transmitted from bats to humans.

Viral neutralizing antibodies in the sera of vaccinees reflect protective immunity against rabies. However, after some time the antibodies may vanish, although there is still a protective immunity as is known from dog challenge tests. This process is not well understood and research continues. Recent studies have demonstrated that in addition to the rabies glycoprotein, the internal nucleocapsid protein of the rabies virus also plays an important role in the induction of protective immunity in laboratory rodents and in cynomolgus monkeys.

Recent studies in rats have demonstrated that it is the rabies glycoprotein that determines the distribution pattern of rabies virus in the brain.[25] Studies such as this are necessary for further understanding of rabies pathogenesis and for new vaccine development.

Vaccine development continues to improve the immunization process for rabies. The state-of-the-art human rabies vaccine -- the human diploid cell vaccine (HDCV) -- is a vast improvement over earlier vaccines. However, it still has some important shortcomings. Unfortunately, the cost of immunization with this vaccine is very high, making it unavailable for large-scale immunization programs in developing countries. In addition, HDCV is made from fixed rabies virus strains, which are carefully standardized and have the same, unchanging chemical makeup. The wild strains of virus that actually infect people and animals may not be a perfect match between their antigens and those of the fixed strains in the vaccine. Allergic reactions to the vaccine occur in about six percent of people who have been vaccinated and later given booster shots. Generally, the allergy is to protein impurities in the vaccine and not to the rabies virus itself.

The eventual elimination of rabies is rooted in control of the disease in

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animals, particularly in dogs. While safe and effective vaccination programs have decreased the threat of rabies in companion animals in the U.S., Canada and Europe, research continues to find more effective rabies control mechanisms that are viable solutions to canine rabies in the developing world.

The control of rabies in wildlife, certain species in particular, remains difficult. Research is still necessary for the development of more effective technology for wildlife immunization against rabies.

What lies ahead in rabies research?

More than a century after Pasteur's original rabies vaccine, scientists have a lot more knowledge to work with in improving rabies prevention and treatment. Not only has the virus been isolated and observed through electron microscopes, but researchers have mapped the surface of the virus particles and worked out the chemical structure of some of its key components. Instead of working blindly through animal or human immune systems, methods can be custom-designed to attack particular parts and abilities of the rabies virus.

Scientists studying rabies today are focusing their efforts on basic research to better understand the virus and its pathogenicity; on developing improved diagnostic techniques; on epidemiological studies; and on vaccine development.

Epidemiological studies today emphasize the epidemiology of "host switching" between wildlife reservoirs of rabies (raccoons, foxes and bats) and domestic animals (dogs, cats and wildlife). Epidemiologists are busy unravelling the public health implications of this phenomenon.

Research continues on development of new diagnostic methodologies for the detection, characterization and quantification of the rabies virus. This includes methods for studying the purification and cultivation of rabies viruses and their components, properties of viral antigens and the production of antibody for diagnostic purposes, and the standardization and harmonization of existing diagnostic procedures.

The development of effective oral vaccines would make treatment less expensive and simpler. Researchers are also trying to make rabies protection broader, to include not only all the circulating rabies strains but also the rabies-related viruses. Another goal is to make protection against rabies longer-lasting, possibly over an entire lifetime.

No single therapeutic agent is likely to be effective against rabies, but a combination of specific therapies are being considered, including rabies vaccine, rabies immunoglobulin, monoclonal antibodies, ribavirin, interferon-alpha, and ketamine. As research advances, new agents may become available in the future for the treatment of human rabies.[26]

The world supply of human rabies immunoglobulin (HIG) for passive immunization against rabies is limited and the product is expensive and not widely available in developing countries. Equine rabies immunoglobulin is a cheaper and more widely available alternative in some countries, but adverse reactions occur more

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frequently than with HIG. Alternative approaches need to be developed for passive immunization of humans at risk. The use of a cocktail of human **monoclonal antibodies** or even a mouse monoclonal antibody may be a promising alternative, and the experimental utility of this approach has recently been explored both ***in vitro***[27] and in animal models.[28,29]

Research is also underway on the use of monoclonal antibodies for post-exposure treatment of humans and animals. Monoclonal antibodies (mABs) have been shown to protect Syrian hamsters against rabies.[30] Although the mABs were of murine origin, mouse antibodies have been used extensively in the treatment of cancer patients without significant side effects. However, recombinant DNA techniques are available to prepare chimeric (mouse-human) antibodies and also to "humanize" mABs of murine origin. Following cloning and sequencing of those "humanized" murine antibodies, it is possible to have them expressed in vectors such as baculovirus.

New Generation Vaccines

The new era of vaccine development based on recombinant DNA (rDNA) technology and other modern techniques will have a great impact on rabies vaccine development. The new technologies promise even more potent, safer vaccines, as well as lower costs, improved stability and easier delivery.

Some researchers have recently shown that the human diploid cell vaccine (HDCV) provides protection against all strains of rabies isolated around the world. HDCV is too expensive, however, to make it a viable immunization option in developing countries. One new vaccine approach is the development of a less costly DNA-based vaccine that would show broad protection against rabies.

DNA vaccines are part of the the new generation of vaccine technology. In 1990, the direct gene transfer of **plasmid DNA** into mouse muscle *in vivo* proved effective for expression of proteins within the muscle cells.[31] This study provided evidence for the idea that naked DNA could be delivered to a living system to direct protein expression. Many subsequent studies have evaluated different factors that determine the efficiency of gene transfer and immunogenicity of plasmid DNA (DNA derived from bacteria). The use of plasmid DNA to immunize against disease is known as DNA vaccination.

DNA vaccines consist of plasmid vectors that contain foreign genes (transgenes) and allow protein expression in mammalian cells. DNA vaccines are stable and not denatured by heat. DNA vaccines are inexpensive, stable and relatively easy to produce and do not need refrigeration. These are qualities that make feasible their widespread use in developing countries. Research continues on rational approaches to DNA vaccine design, including a vaccine producing rabies virus glycoprotein.[32] As DNA vaccine technology advances, its efficiency will increase, making it likely to be a primary method of vaccination in the future.

Increasing knowledge of the molecular biology of plant viruses has raised the possibility of using these viruses as vaccine vectors. Unlike conventional recombinant vaccines, which are largely derived from live-recombinant, live-attenuated or killed pathogens, plant viruses are generally recognized to be non-pathogenic in

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humans and other animals. Thus, interest in plant viruses as vaccine delivery systems is growing. Immunization with recombinant alfalfa mosaic virus containing portions of rabies virus is being studied with mice.[33]

The vaccinia recombinant glycoprotein vaccine (VRG) is now being used to bring rabies epizootics among wild animals under control. The VRG vaccine contains the rabies glycoprotein antigen from the surface of the virus. However, there are other kinds of proteins, nucleoproteins, inside the rabies virus. These "N" proteins do not stimulate the production of antibodies as surface proteins do, but they do stimulate other types of immune defenses. It is believed that they stimulate specialized "killer cells," white blood cells that attack viruses directly. They also prompt the secretion of interferon, a more general defense against viruses that helps to stop the spread of invading pathogens to uninfected cells. And the "N" proteins do not tend to vary among rabies strains as the "G" proteins do, making this a desirable approach to providing broader and more effective protection against rabies.

Researchers are also exploring new virus **vectors**, the delivery vehicle used to carry the viral antigens. The VRG vaccine uses **vaccinia virus** as the vector. Some animals, e.g., skunks and dogs, do not respond well to this vaccine and might be more susceptible to other virus vehicles. Human and animal **adenoviruses** are also sufficiently large to accomodate foreign genes. When inserted into the adenovirus genome, the complementary DNA of rabies virus glycoprotein gene is expressed on the surface of virus particles. Such vaccines would be especially useful for protecting animal species that have been difficult to immunize using existing oral vacciness.[34] Recent studies indicate that the recombinant adenovirus expressing rabies virus glycoprotein is capable of inducing antibody immune responses in dogs and may be developed as a rabies virus vaccine for dogs.[35]

Still another approach is the production of **subunit vaccines**, in which virus proteins (or fragments of them) are used instead of whole viruses to stimulate antibody production. Subunit vaccines prepared from "G" and "N" proteins are showing more promise and are also helping scientists to learn more about how the rabies proteins and the immune defenses against them work.

Rabies vaccines are also being made from synthetic **peptides** - small chains of amino acids produced synthetically to mimic the structure of portions of the natural virus proteins. Both the peptide and subunit vaccines have several advantages. Not only are they fairly inexpensive to produce, they should be safer as well, since no viruses or even any rabies genes are being introduced into the body.

GLOSSARY

Adenovirus... a type of virus that causes common cold-type respiratory illnesses.

Animal model ... an animal used in research that develops or can be induced to develop conditions mimicking a given disease or condition.

Antibody ... a protein that attacks invading pathogens or prevents them from infecting body cells.

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Antigen ... a substance that stimulates the production of antibodies and reacts specifically with them.

Attenuate ... to reduce the virulence, or potency, of a virus.

Cautery... the burning of wound tissues.

Centers for Disease Control... a U.S. Public Health Service agency responsible for assessing the status and characteristics of diseases in the U.S. CDC supports the design, implementation, and evaluation of prevention activities and maintains various public information services.

Clone... a culture of identical cells that share the same heredity.

Dumb rabies ... a form of rabies marked by sluggishness and by early paralysis especially of the muscles of jaw and throat.

ELISA... Enzyme-linked Immunosorbent Assay. A quantitative *in vitro* test for an antibody or antigen.

Encephalitis... inflammation of the brain.

Endemic ... the continuous presence of a disease in a geographic location, community or population.

Epidemic ... a widespread outbreak of a disease, usually in human populations.

Epidemiology ... the study of patterns of disease in human populations. Epidemiological studies provide compelling evidence for measuring environmental risks to people.

Epizootic ... an epidemic of disease among animals. [*Compare with* ***Epidemic***.]

Epizootiology ... the study of patterns of disease in animal populations. [*Compare with* ***Epidemiology***.]

Fixed rabies virus strains... carefully standardized virus strains.

Fluorescent antibody (FA) test ... a test for the rabies virus that uses antibodies against the virus chemically joined with a fluorescent dye that glows under ultraviolet light.

Furious rabies ... a form of rabies characterized by spasm of the muscles of throat and diaphragm, choking, salivation, extreme excitement, and evidence of fear often manifested by indiscriminate snapping at objects .

Glycoproteins ... large molecules containing both protein and carbohydrate portions.

Homologous ... similarity in appearance or structure (though not necessarily in function). Homologous DNA is similar at matched positions. Homologous blood or tissue transfers are those which are transferred or transplanted from one individual to another.

Human Diploid Cell Rabies Vaccine ... a cell culture vaccine against rabies.

Human rabies immunoglobulin... a blood fraction containing antibodies against rabies.

Humane endpoints... in an experiment or study, the earliest indicator in an animal of pain, distress or impending death. Humane endpoints are used to terminate the experiment/study or to begin therapy, thus allowing the animal to be humanely euthanized or to recover as soon as the scientific objective has been achieved.

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Hybridoma... a combination (hybrid) of an antibody-producing cell with a hardy, fast-growing cancer cell; used to produce monoclonal antibodies.

Hydrophobia... a symptom of some rabies cases, literally meaning "fear of water."

Immunization... the administration (orally or by injection) of a pathogen (usually killed or inactivated) or parts of one to stimulate the body's immune defenses against it.

Immunogenic ... relating to or producing an immune response .

Immuno-globulins ... the blood fraction that contains antibodies.

Immunology ... the science involving study of the immune system.

***In vitro* ...** an artificial environment created outside a living organism (eg, in a test tube or culture plate) in experimental research to study a disease or process.

***In vivo* ...** referring to studies conducted within a living organism (eg, animal or human studies).

Incubation period ... the time between infection and the appearance of disease symptoms.

Interferon... a body protein produced in response to virus infection that protects surrounding cells from infection.

***Intra vitam* ...** "during life;" while the subject is still alive.

Lyssavirus ... the virus group to which the rabies virus belongs.

Monoclonal antibodies ... extremely pure antibodies that react specifically with a particular antigen.

Negri bodies ... clumps of virus material containing rabies RNA that accumulate in cells when the virus reproduces.

Neonatal ... relating to a newborn human or animal.

Nucleoproteins ... proteins found in the inner core of a virus, associated with its genetic material.

Oral vaccine ... a vaccine given by mouth.

Passive Immunization ... the introduction of exogenous antibodies (e.g., gamma globulins) to treat disease, for example, from an individual with active immunity, or of genetically-engineered antibodies.

Pathogen ... a specific causative agent (e.g., a bacterium or virus) of disease.

Peptide ... a short chain of amino acids linked by peptide bonds; longer chains are generally called proteins.

Plasmid DNA ... DNA derived from bacteria.

Prophylaxis ... treatment that helps to prevent a disease or condition from occurring or recurring.

Rabid ... showing signs or symptoms of rabies disease.

Rabies Vaccine Adsorbed (RVA) ... a rabies vaccine produced in cultures of rhesus monkey kidney cells.

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Rabies virus neutralizing antibody (VNA)... an antibody against the rabies virus.

Receptor ... a molecule on the surface of a cell that serves as a recognition or binding site for antigens, antibodies, or other cellular or immunological components.

Recombinant DNA... genetically engineered DNA (e.g., new DNA produced by joining pieces of DNA from different sources).

Recombinant vaccine... a vaccine produced by combining a harmless carrier virus (such as vaccinia) with a portion of the genetic material of a pathogen.

Rhabdovirus ... the family of viruses that includes the rabies virus.

RNA ... ribonucleic acid. Structurally similar to DNA, RNA is a nucleic acid, found mostly in the cytoplasm of cells, that is important in the synthesis of proteins. Most forms of RNA (including messenger and transfer RNA) consist of a single nucleotide strand, but a few forms of viral RNA that function as carriers of genetic information (instead of DNA) are double-stranded.

Strain ... a specific genetic variant of a particular organism. Many pathogens have stronger and weaker strains, drug-sensitive and drug-resistant strains, etc.

Subcutaneous ... beneath or introduced beneath the skin (eg, subcutaneous injections).

Subunit vaccine ... a vaccine in which virus proteins or fragments of them are used instead of whole viruses to stimulate antibody production.

Vaccination ... ththe administration (orally or by injection) of a pathogen (usually killed or inactivated) or parts of one to stimulate the body's immune defenses against it.

Vaccine ... a substance that contains antigenic components from an infectious organism. By stimulating an immune response (but not disease), it protects against subsequent infection by that organism.

Vaccinia rabies glycoprotein (VRG) ... a recombinant vaccinia virus containing the rabies glycoprotein gene.

Vaccinia virus ... a relative of the smallpox virus that was used for vaccination against smallpox. Vaccinia is used as a live virus vector is some other vaccines, e.g., oral wlidlife vaccine for rabies.

Vector ... a virus vehicle used as a carrier for delivery of disease pathogen genes in a recombinant vaccine.

Vero cells ... a culture of monkey kidney cells, which can be grown on plastic beads, and give a very high yield of rabies virus for study and vaccine production.

Viral neutralizing antibodies ... an antibody that prevents a virus from infecting a cell, usually by blocking receptors on the cell or the virus.

Virulent ... ability of a virus to cause disease.

Zoonotic disease ... a disease communicable from animals to humans and vice versa.

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