REVIEWARTICLE

Rabies

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ABSTRACT: Rabies is an important disease in wildlife in the United States and Canada, and dog rabies is still a major public health problem in many developing countries of the world. Rabies virus is transmitted in saliva by animal bites. Bats transmitted most recent cases of human rabies in the United States, often without known exposures. There have been recent developments in our understanding of rabies pathogenesis. Characteristic clinical features should raise the possibility of a diagnosis of rabies and initiation of appropriate diagnostic tests. Therapy of human rabies has been futile except in four patients who were immunized with rabies vaccine prior to the onset of their disease. Rabies can be prevented after an exposure in unimmunized patients with local wound cleansing and administration of rabies vaccine and human rabies immune globulin.

RÉSUMÉ: La rage. La rage est une maladie importante chez les animaux sauvages aux Etats-Unis et au Canada et la rage chez le chien demeure un problème important en santé publique dans plusieurs pays en voie de développement. Le virus de la rage est transmis par la salive lors de morsures par des animaux rabiques. La chauve-souris a été le vecteur de la rage chez la majorité des cas récents aux États Unis, souvent sans exposition connue. Il y a des développements récents dans notre compréhension de la pathogenèse de la rage. Les manifestations cliniques caractéristiques devraient faire penser au diagnostic de la rage et inciter à effectuer les tests diagnostiques appropriés. Le traitement de la rage chez l'humain a été futile sauf chez quatre patients qui avaient été immunisés au moyen du vaccin antirabique avant le début de leur maladie. La rage peut être prévenue après une exposition chez les patients non immunisés en nettoyant la plaie et en administrant le vaccin antirabique et de l'immunoglobuline antirabique.

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Rabies is an acute viral infection of the central nervous system caused by rabies virus, which is transmitted in the saliva by biting mammals. Human rabies remains an important disease in the world and rabies mortality ranks about eleventh of all infectious diseases,[1] although it is almost always preventable. Wild animals, including bats, raccoons, skunks, and foxes, are the important rabies vectors in the United States and Canada, while dogs are by far the most important rabies vector in the world. There was an increase in the number of human cases of rabies in the United States during the 1990s with up to six cases per year.[2] There have been only 22 cases of human rabies in Canada since 1924. In 1985 and 2000, there were cases transmitted by bats in Alberta[3] and Quebec (Dr N. Turgeon, personal communication), respectively. The first documented human rabies case in pre-confederation Canada was Charles Lennox, the fourth Duke of Richmond and Governor-in-Chief of British North America, who died in Richmond, Ontario in 1819.[4] Worldwide there are over 30,000 reported human deaths per year from rabies,[5] although the actual number is probably greater than 50,000. This review describes the pathogenesis and clinical features of rabies as well as the relevant epidemiology and strategies for prevention of rabies in the United States and Canada.

PATHOGENESIS

Much of what is known about the pathogenesis of rabies has been learned from studies performed in animal models, usually in rodents.[6] A bite from a rabid animal can result in inoculation of saliva containing infectious rabies virus into subcutaneous tissues and muscles (Figure 1). The precise events during the incubation period of highly variable length are unknown but the virus is thought to be present at or near the site of the bite during most of this period. There is infection of muscle fibers,[7] which may be an obligatory pathogenetic step, and rabies virus binds to nicotinic acetylcholine receptors at the neuromuscular junction.[8] The virus travels towards the CNS (centripetal spread) within motor and sensory axons by retrograde fast axonal transport at a rate of about 50 – 100 mm per day.[9] Recent preliminary evidence indicates that the rabies virus phosphoprotein interacts with dynein LC8, which is important in both actin- and microtubule-based transport in neurons.[10] Neurons are infected in the spinal cord and, subsequently, there is spread from neuron to neuron within axons in the CNS by fast axonal transport along neuroanatomical connections. Many neuronal cell types are infected in a widespread distribution in the CNS, while glial

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infection is usually relatively uncommon.[11] The nicotinic acetylcholine receptor may also be an important rabies virus receptor in the CNS. In 1998 there were reports by two French groups that the neural cell adhesion molecule[12] and the low-affinity p75 neurotrophic receptor ($p75^{NTR}$)[13] are also rabies virus receptors, although a study in a $p75^{NTR}$ knockout mouse model has raised questions about the importance of the p75NTR.[14] The infection of the brain in rabies results in behavioral changes, likely due to infection of neurons in limbic areas, which facilitates transmission by biting in rabies vectors. There is spread of rabies virus away from the CNS (centrifugal spread) along neuronal pathways, particularly involving the parasympathetic nervous system, which are responsible for infection of the salivary glands, skin (nuchal skin biopsy is a useful diagnostic test), heart, and a variety of other organs.[15] Infectious rabies virus is secreted in the saliva of rabies vectors, which is important for transmission to other hosts.

Figure 1: *Schematic representation of the pathogenetic steps after peripheral inoculation of rabies virus: 1) virus inoculated, 2) viral replication in muscle, 3) virus binds to nicotinic acetylcholine receptors at the neuromuscular junction, 4) virus travels within axons of peripheral nerves by fast axonal transport, 5) replication in motor neurons of the spinal cord and local peripheral sensory (dorsal root) ganglia and rapid ascent to the brain, 6) infection of brain neurons with neuronal dysfunction, and 7) centrifugal spread along nerves to salivary glands, skin, cornea, and other organs. Adapted with permission from Robinson PA (1991) Rabies virus. In:* Textbook of Human Virology, *Second Edition. Belshe RB (ed.), Mosby-Year Book, St. Louis. pp. 517-540.*

Clinical features

The vast majority of cases of rabies are transmitted by a bite. Non-bite exposures include contamination of an open wound, scratch, abrasion, or mucous membrane by saliva or CNS tissue from an infected animal. Transmission to humans by inhalation of aerosolized rabies virus in caves containing millions of bats or in laboratory accidents has been shown and also by transplantation of rabies virus-infected corneas.[6] The incubation period usually lasts for 20 to 90 days, but may rarely last for a few days or for more than one year.[16] Malaise, anorexia, and nausea are early prodromal symptoms. The earliest specific neurologic systems include paresthesias, pain, or pruritus at the site of the healed bite wound, which may reflect infection in local peripheral sensory ganglia related to the site of viral entry. There are two clinical forms of rabies: classical or encephalitic or furious in about 80% of cases and paralytic or dumb in about 20%. In both forms of the disease, consciousness is preserved until much later in the clinical course than in encephalitides caused by most other viruses, which accounts for the characteristic clinical features of the disease. In the classical form there are periods of hyperexcitability typically lasting minutes that are separated by lucid periods. There are features of autonomic dysfunction, including hypersalivation, piloerection (gooseflesh), cardiac arrhythmias, and priapism. Hydrophobia occurs in about 50 to 80% of patients and is likely due to selective infection of neurons that inhibit inspiratory neurons near the nucleus ambiguus, resulting in exaggeration of defensive reflexes that normally protect the respiratory tract.[17] On attempts to swallow there are contractions involving the diaphragm and inspiratory muscles lasting 5-15 seconds. This frequently becomes a conditioned reflex and even the sight of water can result in the muscle contractions. Coma and multiple organ failure develop as the disease progresses.[18] Death usually occurs within two weeks, although it may be delayed if patients are managed aggressively in an intensive care setting. Survival has only occurred in four well-documented cases where patients received rabies immunization prior to the onset of their neurologic disease.[19-23]

Laboratory investigations

Although there are few reports of imaging studies, CT and MR imaging of the brain have generally been reported not to show specific abnormalities in rabies. Similarly, electro-encephalography usually shows only nonspecific abnormalities and is not diagnostically helpful. CSF analyses may show a mononuclear pleocytosis, especially after a few days of the neurologic illness. Anderson et al[24] found a CSF pleocytosis in 59% of patients in the first week of illness and in 87% after the first week. Infectious rabies virus has only rarely been cultured from CSF. Rabies virus infection can be diagnosed serologically based on the presence of serum neutralizing antibodies against rabies virus in previously unvaccinated patients, but these antibodies are not usually present until the second week of illness and they may not be present even by the time of death.[18,24] Rabies virus antigen may be detectable in tissues using the direct fluorescent antibody staining technique. Diagnostic skin biopsies taken from the nape of the neck may demonstrate the presence of rabies virus antigen in small nerves adjacent to hair follicles.[25]

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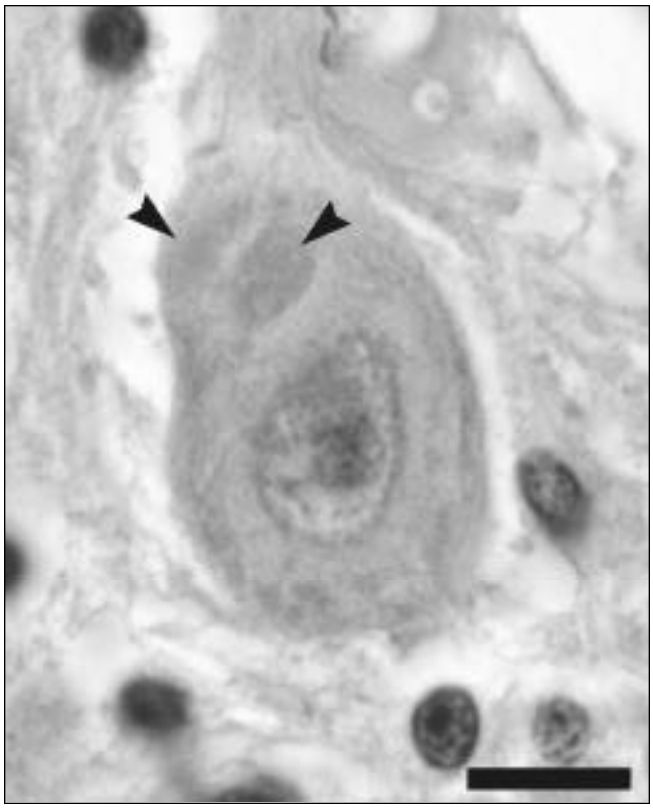

Figure 2: *Cerebellar Purkinje cell from a fatal case of human rabies. Eosinophilic inclusions called Negri bodies (arrowheads) are present in the cytoplasm. Hematoxylin and eosin. Bar=10 μm.*

Rabies virus antigen can be less reliably detected in corneal impression smears.[2] Infectious rabies virus may be isolated from saliva. Detection of rabies virus RNA using reverse transcription-polymerase chain reaction (RT-PCR) amplification is a relatively new advancement in the antemortem diagnosis of rabies.[26] Using this technique, rabies virus RNAmay be detected in saliva, skin biopsies, CSF, and brain tissue. Rabies virus RNA was detected in saliva in ten of ten patients from the United States who were assessed with this technique since 1980.[2]

PATHOLOGY

Despite the dramatic neurological illness in rabies, the pathology is usually relatively bland.[6] There are inflammatory infiltrates in the leptomeninges and parenchyma, including perivascular cuffing with mononuclear cells and microglial nodules (Babes' nodules).[27] Frequently, characteristic eosinophilic cytoplasmic viral inclusions called Negri bodies (Figure 2) are found in the cytoplasm of infected neurons, and ultrastructural studies have shown that they consist of viral particles embedded in an amorphous or slightly electron-dense matrix containing viral proteins.[28] However, Negri bodies are not found in all cases of rabies. Degenerative neuronal changes are not usually prominent, which suggests that neuronal dysfunction of unknown cause is likely important in the pathogenesis of rabies.[6] Rabies virus infection has been associated with apoptosis both in cell culture and in neurons in rodent models under specific experimental conditions.[29-34] In some experimental situations apoptosis of virus-infected neurons may be an "altruistic" response and serve to limit viral spread in the host,[35] while in other situations apoptosis of virus-infected neurons may be detrimental to the host and lead to increased neuronal injury. However, the relevance of observations in experimental models of apoptosis to natural rabies, in which features of neural apoptosis are not prominent, is not clear.

EPIDEMIOLOGY

The vast majority of the 50,000 or more human rabies deaths per year in developing countries, where dog rabies is endemic, are due to transmission of rabies virus from dogs.[5] In the United States and Canada rabies vectors are primarily sylvatic and include bats, raccoons, skunks, and foxes.[36] Terrestrial vectors of rabies have a well-characterized geographic distribution (Figure 3), while bat rabies is present in every state in the US except Hawaii. Over the last few decades a raccoon rabies epizootic has spread north in the eastern United States and during 1999 it crossed the New York border into Ontario, Canada.[37] For reasons that are unclear, there has not been a reported human case of rabies with transmission from a raccoon. Of the 37 cases of human rabies in the US since 1980, 12 cases were imported with transmission from dogs and 25 cases were indigenously acquired, with transmission from bats in 22 of the 25 cases.[2] The majority of the bat – transmitted human cases have been ascribed to a rabies virus variant associated with silver-haired bats (*Lasionycteris noctivagans*) (Figure 4) and eastern pipestrelle bats (*Pipistrellus subflavus*).[2] There was a history of a known untreated bite in one of these cases but no available history of contact with bats in most of the other cases. In these cryptic exposures, the trauma was likely minimal from the bites of these bats, leaving individuals unaware that they were bitten. In comparison with other rabies virus strains, the silver-haired bat rabies virus strain has been shown to replicate well at lower than normal body temperatures (34°C) and has higher infectivity in

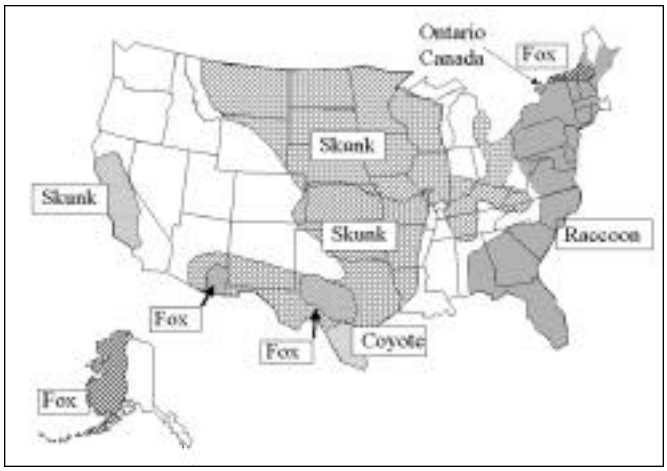

Figure 3: *Geographic distribution of terrestrial vectors of rabies in the United States. From Centers for Disease Control and Prevention.*[36]

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Figure 4: *A silver-haired bat (*Lasionycteris noctivagans*), which is an important vector for transmission of rabies virus to humans in the United States. The rabies bat virus variant in silver-haired bats is also present in eastern pipistrelle bats (*Pipistrellus subflavus*). Both of these species of bats are present in the United States and Canada. Copyright Dr. M. Brock Fenton, York University, North York, Ontario.*

cell types present in the dermis, including fibroblasts and epithelial cells, indicating that it has biological and biochemical properties associated with adaptation for initiating infection after superficial exposures.[38]

Control of rabies in wildlife remains an important challenge for government officials. Until recently, rabies was a problem in red foxes in southern Ontario, in gray foxes in western Texas, and in coyotes in southern Texas.[39,40] Oral vaccination programs involving aerial bait distribution methods have shown promise in the control of fox rabies in these areas and also allowed Switzerland, which had rabies in red foxes, to be declared free of rabies in 1999.[39] Vaccination programs against raccoon rabies, including both trap-vaccinate-release programs and oral vaccination projects using a vaccinia-rabies virus glycoprotein recombinant virus vaccine, have also been deployed and it remains to be seen how effective they will be in controlling the raccoon rabies epizootic in the United States and Canada.[37,39]

Treatment

Therapy of rabies has been unsuccessful except in four reported cases in which rabies vaccination was completed prior to the onset of the disease.[19-23] In all other reported cases, both antiviral therapy with ribavirin and immunotherapies with anti-rabies virus hyperimmune serum or interferon-alfa have been unsuccessful. Therapy is supportive and adequate sedation and analgesia are very important for palliation. In order to be effective, preventative therapy must be initiated after exposures in order to prevent rabies.

Prevention

A variety of animal control measures, including dog control and vaccination programs, are important for the prevention of rabies in humans. Rabies can be prevented in humans following exposures if current guidelines from the Advisory Committee on Immunization Practices are followed. These guidelines are published in the Morbidity and Mortality Weekly Report[41] and are also available on the Internet at http://www2.cdc.gov/mmwr. Even minor deviations from the guidelines have resulted in failure of postexposure prophylaxis (PEP) and the development of rabies. For example, administration of rabies vaccine in the gluteal region (containing adipose tissue) instead of the deltoid muscle may result in low rabies virus neutralization titers and has been associated with a fatal case of rabies.[42-44] A decision must be made as soon as possible that an exposure carries a risk of rabies virus transmission. Details about the species of the animal, clinical status of the animal, and local epidemiology are important in this assessment. Local public health officials are a valuable source of information in reaching a decision about management of an individual. Healthy dogs, cats, and ferrets with normal behavior may be observed for a period of ten days after an exposure and if the animal shows no signs of rabies, then PEPdoes not need to be initiated because the animal's saliva can be presumed to not contain infectious virus. However, this is not the case for other animal species and prophylactic therapy should not be withheld during an observation period because infectious rabies virus may be excreted in their saliva for many days before they develop neurologic signs or abnormal behavior. Laboratory testing for rabies virus antigens on the brain of a suspected rabid animal performed using the fluorescent antibody and culture techniques can provide confirmation of whether an animal has rabies so that appropriate preventive treatment can be initiated.

PEP includes wound cleansing and both active and passive immunization.[41,45] Wounds should be washed immediately and thoroughly with soap and water. A virocidal agent may also be used, especially for deep wounds. Passive immunization of unimmunized patients should include inoculation of 20 IU per kg of human rabies immune globulin with as much of the dose as anatomically feasible inoculated directly into and around the wounds and the remainder of the dose administered intramuscularly into the gluteal muscles or lateral thigh muscles (in young children). Five 1 ml doses of rabies vaccine should be given intramuscularly in the deltoid muscle on days 0, 3, 7, 14, and 28. Human diploid cell vaccine, purified chick embryo cell culture vaccine, and rabies vaccine adsorbed are effective rabies vaccines that are currently licensed in either the United States or Canada. Neutralizing antibodies against rabies virus appear

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about seven to ten days after vaccination. Mild systemic or local side effects may occur after administration of either rabies immune globulin or rabies vaccine, but serious side effects are very unusual.

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