Chapter

The Diagnosis, Clinical Course, Treatment, and Prevention of the Rabies Virus

Jaida Hopkins, Samantha Sweck and Sean Richards

Abstract

Rabies, despite available vaccines, causes approximately 55,000 deaths every year. Diagnosing relies on noting physical behaviors such as hydrophobia, vomiting, fever, behavior changes, paralysis, and consciousness, as well as, using several methodologies to molecularly detect the presence of the virus. RABV often enters through a bite wound given that it is transmissible through saliva. Infection spreads from muscle fibers into the peripheral nervous system traveling to the central nervous system. Infection of the central nervous system can lead to encephalitis (furious rabies) or acute flaccid paralysis (paralytic rabies). Treatment relies heavily on the time of exposure. If the patient is diagnosed prior to being symptomatic, post-exposure prophylaxis (PEP) can be administered. However, once the patient has begun displaying symptoms, therapy success rates sharply decline. Prevention includes vaccinating during both pre- and post-exposures, as well as utilizing Stepwise Approach towards Rabies Elimination (SARE) to aid impoverished countries in declining their rabies mortality rates.

Keywords: rabies virus, RABV, diagnosis, treatment, prevention

1. Introduction

Rabies, an RNA virus from the genus Lyssavirus, also known as RABV, is a lethal pathogen to several species [1]. The evolutionary history of the virus suggests that its most recent ancestor likely diverged into two descendants, one infecting bats, the other infecting dogs. Once domesticated dogs became infected in the Old World, humans became the next target. Evolutionary biology tells us that rabies likely did not exist in the New World prior to the settlement of Europeans but was common throughout Europe, Asia, and Africa well before the discovery of the Americas [2].

RABV is transmitted through the saliva of an infected individual into the bloodstream of a healthy individual, typically via a bite, but possible through other wounds or ocular route. Upon infection, there are two ways the virus may manifest: furious and paralytic. Furious rabies often presents itself with bouts of anxiety, irritability, phobias, and many other symptoms that will later be discussed. Paralytic rabies has some common symptoms with furious rabies, however, paralysis is the most notable symptom just prior to death [3].

According to the CDC in 2015, nearly 60,000 human deaths occurred on average each year. Statistically, this can be interpreted as 1 human death every nine

minutes [4]. Despite its large impact globally, the virus is relatively diminutive. The rabies virus has evolutionarily reduced its single-stranded RNA genome to only five genes. These genes encode five proteins, three of which make up the ribonucleoprotein (RNP) complex; the other two form the virus's envelope [5].

While the rabies virus has been heavily researched, lives continue to be lost. There is a great understanding of the epidemiology of the virus, however little is understood about the pathogenesis. Herein, the findings regarding the diagnosis, clinical course, treatment, and prevention of the rabies virus are summarized as well as data gaps in research and understanding of this pathogen.

2. Diagnostics

Once the patient has become infected, the incubation period can be as short as 5 days or as long as 2 years. However, it is common for symptoms to occur 20–90 days after initial exposure [6]. Nevertheless, there are several techniques for diagnosing a patient during and after the incubation period.

2.1 Physical symptoms

As mentioned previously, the two ways the rabies virus may present itself is paralytic and furious symptoms. A patient with paralytic rabies will show progressive paralysis until death. If infection occurred due to a bite, the paralysis typically starts around the wounded area, spreading outwards. It is also common for patients to have a fever, vomiting, weakness in muscles, and myalgia prior to the paralysis [7].

Furious rabies often presents itself with more obvious symptoms. The infected individual commonly displays mood swings, unregulated consciousness, phobias, especially hydrophobia, as well as spasms of the respiratory system [1, 8]. Other symptoms may include a cough, psychosis, delirium, and difficulties swallowing [9].

2.2 Neuroimaging

For both furious and paralytic rabies, magnetic resonance imaging (MRI) will produce the same key diagnostic images. When symptoms first arise in the prodromal phase, progression in hypersignal T2 changes can be seen around the brachial plexus and the spinal nerve roots associated with the extremity of infection origin. The main MRI feature is increased T2 signal (seen on T2 and FLAIR sequences) in the affected parts of the brain and spinal cord, with a predilection for grey matter structures including basal ganglia, thalami, hypothalami, limbic system, and brainstem. The abnormal hypersignal T2 changes will continue to progress as the patient enters a coma. Once in the comatose phase, the contrast will enhance around the spinal cord, nerve roots located at the sine and cranial region, deep grey matter, brain stem, limbic structures, and thalamus (**Figure 1**) [8].

Another study noted that lesions could be seen throughout different areas of the neuroaxis. They, too, found that paralytic and furious rabies present the same MRI indications, however, they are more noticeable in the paralytic form. The blood–brain barrier often shows no sign of damage until the patient reaches the comatose state. Imaging of the blood–brain barrier has greatly improved as new techniques such as diffusion-weighted and diffusion tensor imaging can capture objective and subjective data [11].

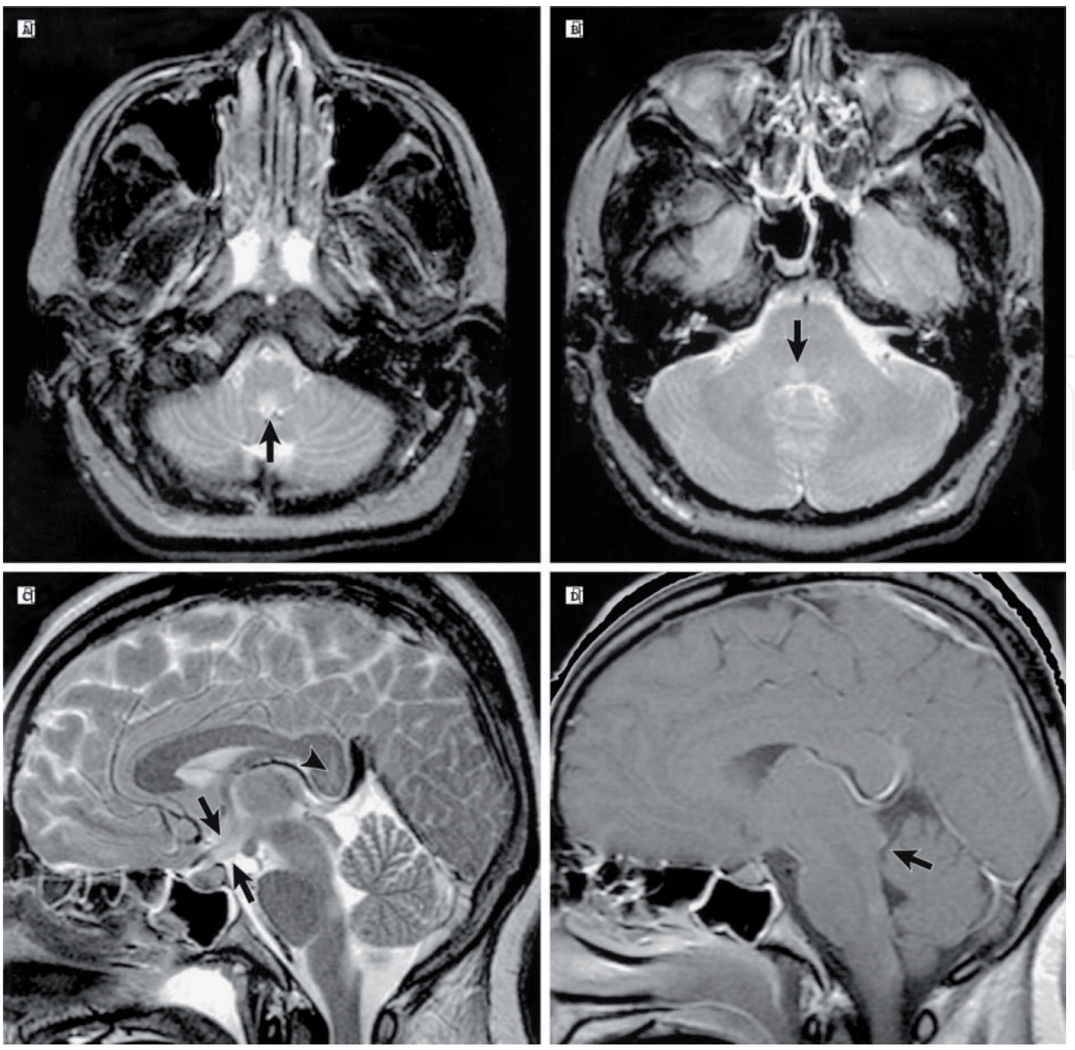

Figure 1.
Magnetic resonance images with arrows pointing to high focal areas (A. dorsal medulla, B. pons, C. hypothalamus, and D. splenium of corpus callosum) of an infected patient with furious rabies [10].

2.3 Testing techniques

There are several techniques used to diagnose a patient with a rabies infection; including reverse transcription polymerase chain reaction (rtPCR) analysis, fluorescent antibody test (FAT), tissue culturing, and viral antibody neutralization [12, 13]. The FAT assay has long been a microbiological standard for diagnosing rabies (and other viral infections). Fluorescent antibody virus neutralisation (FAVN) test is also used to diagnose rabies [13]. The CDC recommends a direct form of FAT to detect the rabies virus in animals ante-mortem, however, the animal is usually euthanized after detection and brain tissue samples taken to solidify the diagnosis post-mortem. However, diagnosis in humans requires several different types of methods such as direct rapid immunohistochemistry testing, the use of electron microscopy, and reverse transcriptase-polymerase chain reactions on biological samples (**Figure 2**) [14].

Another test utilizing immunohistochemistry, known as the direct rapid immunohistochemistry test (dRIT) is a specific form of histology because it employs antibodies unique to RABV. While this testing method can give a reliable result in less than an hour, retrieving brain samples is very invasive [16].

Samples can also be viewed using electron microscopy. When Negri bodies are located in samples, electron miscopy can give a clear depiction of the bullet-shaped

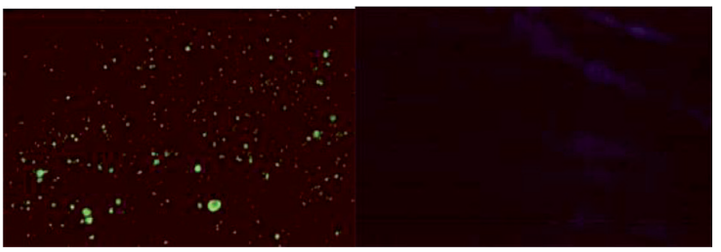

Figure 2.
These images show the comparison of a positive direct FAT result (left) and a negative direct FAT result (right) [15].

rabies virus being produced. A colloid can be used to compare the virus size to further interpret the image, however shape and size alone are not enough to identify with confidence [17].

Saliva and skin samples can be used for identifying the presence of RABV. rtPCR can be used to confirm or oppose the results of FAT test results. Because RABV is a single-stranded RNA virus, rtPCR can help transform RNA into DNA through amplification for analysis of a complement to a rabies-specific primer. This is often achieved by inoculating suckling mice and retrieving brain or kidney samples after death [14, 18].

2.4 Patients with psychiatric disorders

Given that the rabies virus significantly impacts the central nervous system, it is not uncommon for hallucinations, manic episodes, anxiety, and bouts of paranoia to be present in an infected patient [19, 20]. However, these are also common symptoms of psychiatric disorders such as schizophrenia. While it is rare, it is possible that correct RABV diagnosis may be complicated by a previous diagnosis of psychiatric disorders. Patients suffering from hypochondriasis could elicit psuedorabies [20]. In several case studies with reports of hypochondriasis, patients did not respond to therapies and often attempt suicide multiple times [20, 21]. While these situations are rare, they should be noted when diagnosing patients with certainty.

3. Clinical course

3.1 Pathophysiology

The most common entry point for RABV is through the bite of an infected individual as rabies resides in saliva [22]. However, transmission from organ transplants and aerosol droplets have also been recorded [23–25]. Upon entry, an incubation period often takes place in the myocytes, and rarely fibrocytes, where the virus attaches to the G-protein of the cells, enters through pinocytosis or fusion, and replicates in the cytoplasm of the cells [5, 24]. Virions will replicate with little to no immune response until the virus interacts with and infects a nerve cell. The rabies virus gains access to the nervous system by binding to the nicotinic acetylcholine receptors on the postsynaptic membrane at the neuromuscular junction [26]. From the point of nerve cell infection, the virus will travel via axon transport through the nervous system, eventually reaching the brainstem to give rise to either encephalitis or acute flaccid paralysis [7, 27]. The incubation periods are highly variable depending on the dosage of the virus. However, in humans, the incubation period is often >1–3 months. Canine incubation periods are most commonly less than 60 days [26, 28].

Once infection is recognized by the immune system, cytokine, IgM, and IgG antibody production increases [5]. Specific cytokines, Interleukin-1β (IL-1β) and TNFα, are thought to be the reason inflammation of the central nervous system occurs post-exposure [29]. The presence of these cytokines initiate a cascade reaction, upregulating proteins necessary for the inflammatory response such as the major histocompatibility complex and adhesion molecules. These proteins then interact with leukocytes to allow the blood brain barrier to become more permeable, thus causing an immune response leading to encephalitis [30]. As for the natural defense against rabies, RABV signals a series of molecular cascades that initiates type I interferon responses that have antiviral properties, decreasing the pathogenicity of rabies [31]. However, to artificially aid the natural response, there is heavy research on the potential to activate dendritic cells which subsequently enhance the activity of interleukin proteins and high mobility group box 1 (HMBG1) to enhance immunogenicity [32, 33].

4. Treatment

Although the rabies disease has been documented for thousands of years, it is still largely considered incurable after the onset of symptoms [34]. This is due to the small window of time during which aggressive treatment is practical and effective. With that knowledge, most often treatment resolves to be mostly palliative with aggressive treatment proving to be essentially ineffective after RABV is established in the patient.

4.1 Aggressive approach

If a patient presents for treatment early in the process of the clinical disease, the choice may be made to apply an aggressive approach. At this point, the patient must be immediately admitted to an intensive care hospital and post-exposure prophylaxis (PEP) should be administered [26]. PEP consists of immediate washing of the wound with soap and water, a post-exposure vaccination, and injection of an anti-rabies immunoglobulin (RIG) directly into the wound [35].

Active immunization has evolved greatly since the first rabies vaccine was developed and administered in 1885 [36, 37]. Louis Pasteur is credited with this first vaccine that consisted of injecting the patient with homogenates of RABV-infected rabbit spinal cord multiple times over a period of days. The initial injection was believed to be fully inactivated after an extended desiccation period, and each subsequent inoculation was increasingly more virulent as the desiccation period was decreased. While this was found to be somewhat effective, two major issues presented themselves. First, the inactivation of the RABV was inconsistent which led to some patients becoming infected after receiving the vaccine. Second, there was an inequity in the supply of the RABV-infected rabbits and the demand of the human population. These were rectified with the introduction of RABV-infected sheep and goat brain vaccines [36, 37]. These new vaccines were inactivated via chemical agents such as phenol; this proved to be much more consistent. However, soon it was understood that vaccines produced from mature brain material contained an excess of myelin which caused sensitization and ultimately killed the patients [38]. From this discovery emerged the current methodology by which rabies vaccines are created. Chick embryos served the same role as the previous tissues, but they have markedly less myelin due to the young age [39]. The same can be said of the lines of human diploid cells infected with fixed RABV for vaccines [36, 37]. In the United States, there are two CDC-approved vaccines: the human diploid cell culture vaccine (HDCV) and the purified chick embryo cell culture vaccine (PCECV) [14].

The post-exposure vaccine is primarily an intramuscular vaccine that ought to be given on day 0, 3, 7, 14, and 30 [36]. An intradermal vaccine has recently been developed in an attempt to decrease the amount of vaccine needed per injection [40]. While the decreased cost of the vaccine is an attractive option, intradermal injections are generally considered more difficult which could decrease the effectiveness of the vaccine due to improper injections. The World Health Organization (WHO) strongly recommends purified cell culture and embryonated egg-based vaccines [41].

Although the vaccines have been proven to stimulate an appropriate immune response to RABV, this immune response is often too delayed to prevent the virus from entering the nerves [42]. With this knowledge, passive immunization is recommended for all patients that have no previous history with the disease or a pre-exposure vaccination. At this time, two types of rabies immunoglobulin are available – human rabies immunoglobulin (HRIG) and equine rabies immunoglobulin (ERIG). These have been used since the 1970's to temporarily increase the concentration of rabies virus neutralizing antibodies (RVNA) specifically at the site of exposure [43]. If the patient has multiple bites or wounds, the RIG needs to be applied at each site to be effective [44]. HRIG is to be administered at a dosage or 20 IU/kg while the recommended dosage for ERIG is 40 IU/kg [42, 44]. Due to higher odds of sensitization as well as quicker elimination of the RVNA, HRIG is generally the most preferred option for passive immunization. However, it is approximately five times more expensive than ERIG. This results in a major deficiency in many of the countries most in need of rabies treatment [44].

Because the available RIGs are generally inaccessible to developing countries, WHO encouraged researchers to pursue synthetization of a human monoclonal antibodies (mAbs) cocktail that can be used to treat rabies [45]. At this time, there are a number of rabies mAbs products in clinical trials, but none have been approved by the US Food and Drug Administration [46]. In 2018, WHO officially adjusted their recommendations for treatment of rabies to include mAbs products in place of RIG if available [46].

A protocol known as the Milwaukee Protocol was presented as a potential cure in the late 20th century, but it has since been proven to be inconsistent and generally ineffective in reverting or curing the patient of the disease [47–49]. This protocol consisted of an induced coma and one or more antivirals. The coma was soon determined to be ineffective. It is now recommended that sedation should be limited to prevent the use of ventilatory support if possible [48, 49].

Antivirals could serve an important role in the treatment of the rabies virus as they act as inhibitors of viral replication. An effective antiviral could slow the progression of the rabies virus enough for the patient's innate immune response to develop and react appropriately. However, at this time, there are few antivirals supported for the treatment of RABV. Ribavirin is a purine analogue that acts as an RNA mutagen and has shown to be clinically effective for multiple viruses including Hepatitis C and Lassa fever virus [50, 51]. Despite its inclusion in the sporadically effective Milwaukee Protocol, it has repeatedly proven to be ineffective against the rabies virus. Interferon-α (IFN-α) is a signaling protein used to trigger an immune response which has been shown to limit RABV spreading in mice trials [50, 51]. However, additional trials in which primates were administered intramuscular and intrathecal IFN-α determined the effects of immediate inoculation to be incomplete while the effects of delayed inoculation were nonexistent [52, 53]. Six human patients have been treated with IFN-α on two different dosage schedules and despite evidence of increased IFN in serum and CSF, there was no evidence of a beneficial effect on the disease itself [54]. Another therapy previously included in the Milwaukee Protocol is ketamine [50, 51]. At low concentrations, approximately

1 μM, ketamine works as a non-competitive antagonist of the N-methyl-D-aspartate (NMDA) receptor which causes a state of dissociative anesthesia. A study from 1991 showed that a high concentration of ketamine could induce inhibitory effects on the RABV genome transcription [55]. Since that time, multiple other trials using neuron cultures and infected mice have produced evidence that ketamine is generally ineffective against the disease [56]. With that knowledge, it is believed that ketamine should not be used for treatment of the rabies virus until further studies produce more promising results. Amantadine is the third and final antiviral that was considered in the Milwaukee Protocol [47, 51]. It is a synthetic inhibitor of viral replication by impeding the release of viral genetic material into the host cell. Although amantadine demonstrated some interference in cellular trials, it failed in animal trials [57]. Minocycline, a broad spectrum antimicrobial, was considered for RABV treatment as it has proven benefits for multiple other viruses [58]. However, when applied to rabies-infected animals, Minocycline caused a number of harmful effects causing an increase in mortality [59].

More recently, researchers have addressed the use of favipiravir in treatment of rabies [50]. Available for influenza treatment in some countries, favipiravir has shown some activity against the rabies virus in mice trials [60]. Continued studies are needed to assess the future of this and other antivirals. It ought to be noted that despite variations in the effectiveness of these antivirals, all have shown that an early start of treatment greatly improves the efficacy of these therapies.

4.2 Palliative care

As previously mentioned, there is a small window of effectiveness for an aggressive treatment of RABV; after that window has passed, the focus of treatment is purely one of comfort for the patient. Many patients develop phobias that will likely require seclusion in a calm, quiet room. Although there is little to no evidence of human-to-human transmission, visitors and medical professionals need to be cautious of potential contamination via the patient's secretion [61]. Dehydration is a major concern as paralytic rabies often inhibits the patient's ability to swallow and furious rabies can cause intense hydrophobia. Treatment for the dehydration is typically a secured intravenous line. If additional nutrients are needed, they can be administered through the same line.

Rabies typically causes a generalized inflammation that induces a fever, but it can also trigger a neurogenic (central) fever as well [62]. Many antipyretics, such as acetaminophen and ibuprofen, have been successfully used to treat the generalized fever. However, those are generally ineffective towards the central fever. There is some evidence to support the use of baclofen, bromocriptine, chlorpromazine, and morphine in the treatment of a central fever [63]. However, these have not been studied specifically for a rabies-induced central fever and so practitioners should be mindful of potential side effects. Additionally, these drugs are rarely available in the developing countries where RABV is most prevalent [61]. In this case, external, physical means of cooling the patient can be used as needed.

The majority of patients infected with furious RABV develop intense agitation and fear. A variety of sedatives and tranquilizers are used to calm these symptoms. Because benzodiazepines are included on the WHO's list of essential medicines, they are commonly used to treat rabies-induced agitation as well as many other forms [61]. They can be administered intramuscularly, intravenously, or intrarectally as needed. However, as previously mentioned, it is important to administer any sedative slowly to ensure the patient retains consciousness and does not lose respiratory function [64].

Lastly, clinicians will likely need to address the patient's pain level. This can be accomplished using opioids such as morphine or other highly effective analgesics. These can be delivered intravenously, intramuscularly, intrarectally, or even transdermally as needed [61]. Although many of these methods of treatment are expensive and generally unlikely to cure a patient once rabies symptoms are present, palliative care is a responsibility of caretakers and clinicians.

5. Prevention

Due to the general ineffectiveness of post-exposure treatment, the rabies virus has a remarkably high mortality rate despite the availability of vaccines that have shown a near perfect success rate when administered prior to infection. This indicates that the main issue with prevention is the lack of accessibility in the impoverished countries of Asia and Africa. In 2015, the World Health Organization (WHO) and the World Organization for Animal Health (OIE) with the help of the Food and Agriculture Organization of the United Nations (FAO) set a goal to rid the world of dog-mediated rabies by 2030 (Zero by 30) [65]. After assessing cost and general accessibility, it was decided that the best way to eliminate dog-mediated rabies is to vaccinate dogs rather than humans. Vaccinating at least 70% of dogs should effectively break the cycle of rabies transmission.

To reach this goal, WHO, OIE, FAO and many smaller agencies collaborated to create the Stepwise Approach towards Rabies Elimination (SARE) [66, 67]. A similar stepwise approach proved successful in the elimination of fox RABV from Europe [68]. SARE consists of five stages for each country to work through. Stage 0 is simply the lack of information and data on RABV cases in a country where rabies is believed to be present. Stage 1 is an assessment phase in which data are gathered to determine the extent to which RABV pervades the country of interest. During this phase, the government assesses the current guidelines or structures in place as well as collects and analyzes all available data on previous or existing RABV cases. The beginning of an action plan is usually concocted in Stage 1. Evolution of this plan happens in Stage 2; it is important to develop an understanding of the available funding at this point as that has been the biggest limitation for developing countries in the past. Stage 3 is the implementation of the country's rabies control strategy. During this phase, the plan will likely need to be adapted to address any challenges that arise; these may include the exposure of wildlife reservoirs of RABV such as the fox rabies previously found in Europe. When reported human cases have decreased to zero, the country will shift into Stage 4 – elimination of dog RABV. This requires the maintenance of the reduced dog-to-human rabies transmission as well as continued implementation of the action plan to continue to reduce dog rabies cases. Lastly, in Stage 5, the country must develop a post-elimination strategy to maintain the freedom from human and dog rabies.

While the SARE tool has been developed to be adaptable enough to succeed worldwide, there are likely to be some setbacks in the different landscapes. Specifically, areas of poverty will be constrained by the financial resources they can acquire. Many parts of Asia have political instability that will greatly challenge the need for nation-wide commitment to this goal. Similarly, Africa's linguistic and cultural complexity will oppose the need for excellent communication and tracking [66]. Ultimately, it will take a dedicated and educated global population to eliminate rabies as a whole; until then, vaccinating animals and at-risk populations is the most efficient means of prevention.

6. Conclusion

This chapter served to outline the diagnosis, clinical course, treatment, and prevention of the RNA virus RABV. A swift rabies's diagnosis is imperative to ensure the patient's greatest chance of survival. Clinicians may utilize many techniques such as MRI, rtPCR, FAT, FAVN, dRIT, and electron microscopy to diagnose patients before physical symptoms arise. The same methods can be used to confirm a diagnosis after the patient presents with symptoms if needed. Rabies can present in patients in the furious or paralytic form. Although these cause very different physical symptoms, the pathophysiology is very similar. Generally initiated by a bite or other wound, the virus will incubate in the myocytes nearest the entry point and replicate undetected for an average of 1–3 months. Eventually, the virus will infect a nerve cell and travel through axon transport to the CNS at which point it will cause encephalitis or paralysis. Once the virus has infiltrated the CNS, it is essentially incurable. However, if detected early, patients can receive PEP - a combination of active and passive immunization most frequently in the form of a cell culture vaccine and a dose of RIG. At this time, there are no antivirals considered to be effective for treating RABV. If the virus becomes established in the patient, the treatment plan is adjusted to ensure the most comfort for the patient and their loved ones. This most often consists of a variety of sedatives, pain management, antipyretics, and fluids. Due to the great lethality of RABV in humans, multiple global organizations have banded together to attempt to eradicate the modern world of the rabies virus. The Zero by 30 movement strives to implement a stepwise method to eliminate dog-mediated rabies cases worldwide by 2030. This is believed to be possible if 70% or more of the dog population is vaccinated against rabies. In combination with the vaccination goal, Zero by 30 also encourages countries to implement educational standards on rabies, bite prevention, and responsible pet ownership.

At this time, the community of RABV researchers need to be working to improve current treatment options in order to decrease the number of RABV-related deaths. This might be done by developing a mAb alternative to the too-expensive RIG options or finding an effective antiviral either currently on the market or newly developed. The release of additional rabies vaccines for humans would also help to lower the price and improve the accessibility in a way that would benefit the highly affected countries.

Conflict of interest

The authors declare no conflict of interest.

Author details

Jaida Hopkins, Samantha Sweck and Sean Richards*
University of Tennessee, Chattanooga, USA

*Address all correspondence to: seanrichards.utc@gmail.com

References

[1] Jackson AC. Human Rabies: a 2016 Update. Current Infectious Disease Reports. 2016;18(11):38.

[2] Velasco-Villa A, Mauldin MR, Shi M, Escobar LE, Gallardo-Romero NF, Damon I, et al. The history of rabies in the Western Hemisphere. Antiviral Research. 2017;146:221-232.

[3] Mitrabhakdi E, Shuangshoti S, Wannakrairot P, Lewis RA, Susuki K, Laothamatas J, et al. Difference in neuropathogenetic mechanisms in human furious and paralytic rabies. Journal of the Neurological Sciences. 2005;238(1):3-10.

[4] Every 9 Minutes, Someone in the World Dies of Rabies [press release]. Centers for Disease Control and Prevention2015.

[5] Rupprecht CE. Rhabdoviruses: Rabies Virus. 1996. In: Medical Microbiology [internet] [Internet]. Galveston (TX): University of Texas Medical Branch at Galveston. 4th. Available from: https://www.ncbi.nlm.nih.gov/books/NBK8618/.

[6] Jackson AC. Rabies virus infection: An update. Journal of NeuroVirology. 2003;9(2):253-258.

[7] Ghosh JB, Roy M, Lahiri K, Bala AK. Acute flaccid paralysis due to rabies. Journal of pediatric neurosciences. 2009;4(1):33-35.

[8] Hemachudha T, Ugolini G, Wacharapluesadee S, Sungkarat W, Shuangshoti S, Laothamatas J. Human rabies: neuropathogenesis, diagnosis, and management. The Lancet Neurology. 2013;12(5):498-513.

[9] Suraweera W, Morris SK, Kumar R, Warrell DA, Warrell MJ, Jha P, et al. Deaths from Symptomatically Identifiable Furious Rabies in India: A Nationally Representative Mortality Survey. PLOS Neglected Tropical Diseases. 2012;6(10):e1847.

[10] Pleasure SJ, Fischbein NJ. Correlation of Clinical and Neuroimaging Findings in a Case of Rabies Encephalitis. Archives of Neurology. 2000;57(12):1765-1769.

[11] Laothamatas J, Sungkarat W, Hemachudha T. Chapter 14 - Neuroimaging in Rabies. In: Jackson AC, editor. Advances in Virus Research. 79: Academic Press; 2011. p. 309-27.

[12] Robardet E, Picard-Meyer E, Andrieu S, Servat A, Cliquet F. International interlaboratory trials on rabies diagnosis: An overview of results and variation in reference diagnosis techniques (fluorescent antibody test, rabies tissue culture infection test, mouse inoculation test) and molecular biology techniques. Journal of Virological Methods. 2011;177(1):15-25.

[13] Cliquet F, Aubert M, Sagné L. Development of a fluorescent antibody virus neutralisation test (FAVN test) for the quantitation of rabies-neutralising antibody. Journal of Immunological Methods. 1998;212(1):79-87.

[14] Centers for Disease Control and Prevention NCfEaZIDN, Division of High-Consequence Pathogens and Pathology (DHCPP). Rabies [Internet]. Centers for Disease Control and Prevention; 2020 [updated September 25, 2020. Available from: https://www.cdc.gov/rabies/index.html.

[15] Prevention CfDCa, (NCEZID) NCfEaZID, (DHCPP) DoH-CPaP. Direct fluorescent antibody test: Centers for Disease Control and Prevention; 2011 [Available from: https://www.cdc.gov/rabies/diagnosis/direct_fluorescent_antibody.html.

[16] Lembo T, Niezgoda M, Velasco-Villa A, Cleaveland S, Ernest E, Rupprecht CE. Evaluation of a direct, rapid immunohistochemical test for rabies diagnosis. Emerging infectious diseases. 2006;12(2):310-313.

[17] Matsumoto S. Electron microscopy of nerve cells infected with street rabies virus. Virology. 1962;17(1):198-202.

[18] David D, Yakobson B, Rotenberg D, Dveres N, Davidson I, Stram Y. Rabies virus detection by RT-PCR in decomposed naturally infected brains. Veterinary Microbiology. 2002;87(2):111-118.

[19] Goswami U Fau - Shankar SK, Shankar Sk Fau - Channabasavanna SM, Channabasavanna Sm Fau - Chattopadhyay A, Chattopadhyay A. Psychiatric presentations in rabies. A clinico-pathologic report from South India with a review of literature. (0041-3232 (Print)).

[20] Bidaki R, Mirhosseini Mm, Asad F. Rabies or a psychiatric disorder? A rare case report. Neuropsychiatrie de l'Enfance et de l'Adolescence. 2013;60.

[21] Fishbain DA, Barsky S, Goldberg M. Monosymptomatic Hypochondriacal Psychosis: Belief of Contracting Rabies. The International Journal of Psychiatry in Medicine. 1992;22(1):3-9.

[22] Hampson K, Dushoff J, Cleaveland S, Haydon DT, Kaare M, Packer C, et al. Transmission Dynamics and Prospects for the Elimination of Canine Rabies. PLOS Biology. 2009;7(3):e1000053.

[23] Bronnert J, Wilde H, Tepsumethanon V, Lumlertdacha B, Hemachudha T. Organ Transplantations and Rabies Transmission. Journal of Travel Medicine. 2007;14(3):177-180.

[24] Winkler WG, Fashinell TR, Leffingwell L, Howard P, Conomy JP. Airborne Rabies Transmission in a Laboratory Worker. JAMA. 1973;226(10):1219-1221.

[25] Burton EC, Burns Dk Fau - Opatowsky MJ, Opatowsky Mj Fau - El-Feky WH, El-Feky Wh Fau - Fischbach B, Fischbach B Fau - Melton L, Melton L Fau - Sanchez E, et al. Rabies encephalomyelitis: clinical, neuroradiological, and pathological findings in 4 transplant recipients. (0003-9942 (Print)).

[26] Jackson AC, Warrell MJ, Rupprecht CE, Ertl HCJ, Dietzschold B, O'Reilly M, et al. Management of Rabies in Humans. Clinical Infectious Diseases. 2003;36(1):60-63.

[27] Hemachudha T, Laothamatas J, Rupprecht CE. Human rabies: a disease of complex neuropathogenetic mechanisms and diagnostic challenges. The Lancet Neurology. 2002;1(2):101-109.

[28] Charlton KM, Nadin-Davis S Fau - Casey GA, Casey Ga Fau - Wandeler AI, Wandeler AI. The long incubation period in rabies: delayed progression of infection in muscle at the site of exposure. (0001-6322 (Print)).

[29] Marquette C, Van Dam A-M, Ceccaldi P-E, Weber P, Haour F, Tsiang H. Induction of immunoreactive interleukin-1β and tumor necrosis factor-α in the brains of rabies virus infected rats. Journal of Neuroimmunology. 1996;68(1):45-51.

[30] Appolin?rio CM, Allendorf SD, Peres MG, Ribeiro BD, Fonseca CvR, Vicente AcF, et al. Profile of Cytokines and Chemokines Triggered by Wild-Type Strains of Rabies Virus in Mice. The American Society of Tropical Medicine and Hygiene. 2016;94(2):378-383.

[31] Chopy D, Detje Cn Fau - Lafage M, Lafage M Fau - Kalinke U, Kalinke U Fau -

Lafon M, Lafon M. The type I interferon response bridles rabies virus infection and reduces pathogenicity. (1538-2443 (Electronic)).

[32] Wang Z, Liang Q, Zhang Y, Yang J, Li M, Wang K, et al. An optimized HMGB1 expressed by recombinant rabies virus enhances immunogenicity through activation of dendritic cells in mice. Oncotarget. 2017;8(48): 83539-83554.

[33] Chen T, Zhang Y, Wang Z, Yang J, Li M, Wang K, et al. Recombinant rabies virus expressing IL-15 enhances immunogenicity through promoting the activation of dendritic cells in mice. Virologica Sinica. 2017;32(4):317-327.

[34] Tarantola A. Four Thousand Years of Concepts Relating to Rabies in Animals and Humans, Its Prevention and Its Cure. Tropical medicine and infectious disease. 2017;2(2):5.

[35] Organization WH. Frequently asked questions about rabies for the General Public [PDF]. 2018 [Available from: https://www.who.int/rabies/Rabies_General_Public_FAQs_21Sep2018.pdf.

[36] Hicks DJ, Fooks AR, Johnson N. Developments in rabies vaccines. Clinical and experimental immunology. 2012;169(3):199-204.

[37] Wu X, Smith T, Rupprecht C. From brain passage to cell adaptation: The road of human rabies vaccine development. Expert review of vaccines. 2011;10:1597-1608.

[38] Almeida RG, Pan S, Cole KLH, Williamson JM, Early JJ, Czopka T, et al. Myelination of Neuronal Cell Bodies when Myelin Supply Exceeds Axonal Demand. Current Biology. 2018;28(8):1296-305.e5.

[39] Koprowski H Fau - Cox HR, Cox HR. Studies on chick embryo adapted rabies virus; culture characteristics and pathogenicity. (0022-1767 (Print)).

[40] Madhusudana SN, Mani RS. Intradermal vaccination for rabies prophylaxis: conceptualization, evolution, present status and future. (1744-8395 (Electronic)).

[41] World Health Organization = Organisation mondiale de la S. Weekly Epidemiological Record, 2018, vol. 93, 16 [full issue]. Weekly Epidemio logical Record = Relevé épidémiologique hebdomadaire. 2018;93(16):201-20.

[42] Matson MA, Schenker E, Stein M, Zamfirova V, Nguyen H-B, Bergman GE. Safety and efficacy results of simulated post-exposure prophylaxis with human immune globulin (HRIG; KEDRAB) co-administered with active vaccine in healthy subjects: a comparative phase 2/3 trial. Human Vaccines & Immunotherapeutics. 2020;16(2): 452-459.

[43] Aoki FY, Rubin Me Fau - Friesen AD, Friesen Ad Fau - Bowman JM, Bowman Jm Fau - Saunders JR, Saunders JR. Intravenous human rabies immunoglobulin for post-exposure prophylaxis: serum rabies neutralizing antibody concentrations and side-effects. (0092-1157 (Print)).

[44] Bharti OK, Madhusudana SN, Wilde H. Injecting rabies immunoglobulin (RIG) into wounds only: A significant saving of lives and costly RIG. Human vaccines & immunotherapeutics. 2017;13(4):762-765.

[45] Goudsmit J, Marissen We Fau - Weldon WC, Weldon Wc Fau - Niezgoda M, Niezgoda M Fau - Hanlon CA, Hanlon Ca Fau - Rice AB, Rice Ab Fau - Kruif Jd, et al. Comparison of an anti-rabies human monoclonal antibody combination with

human polyclonal anti-rabies immune globulin. (0022-1899 (Print)).

[46] Sparrow E, Torvaldsen S, Newall AT, Wood JG, Sheikh M, Kieny MP, et al. Recent advances in the development of monoclonal antibodies for rabies post exposure prophylaxis: A review of the current status of the clinical development pipeline. Vaccine. 2019;37:A132-A1A9.

[47] Willoughby RE, Tieves KS, Hoffman GM, Ghanayem NS, Amlie-Lefond CM, Schwabe MJ, et al. Survival after Treatment of Rabies with Induction of Coma. New England Journal of Medicine. 2005;352(24):2508-2514.

[48] van Thiel P-PAM, de Bie RMA, Eftimov F, Tepaske R, Zaaijer HL, van Doornum GJJ, et al. Fatal Human Rabies due to Duvenhage Virus from a Bat in Kenya: Failure of Treatment with Coma-Induction, Ketamine, and Antiviral Drugs. PLOS Neglected Tropical Diseases. 2009;3(7):e428.

[49] Hemachudha T, Sunsanee witayakul B, Desudchit T, Suankratay C, Sittipunt C, Wacharapluesadee S, et al. Failure of therapeutic coma and ketamine for therapy of human rabies. Journal of neurovirology. 2006;12:407-409.

[50] Jochmans D, Neyts J. The path towards effective antivirals against rabies. Vaccine. 2019;37(33): 4660-4662.

[51] Appolinário C, Jackson A. Antiviral therapy for human rabies. Antiviral therapy. 2014;20.

[52] Niu X, Tang L, Tseggai T, Guo Y, Fu ZF. Wild-type rabies virus phosphoprotein is associated with viral sensitivity to type I interferon treatment. Archives of Virology. 2013;158(11):2297-2305.

[53] Weinmann E Fau - Majer M, Majer M Fau - Hilfenhaus J, Hilfenhaus J. Intramuscular and/or intralumbar postexposure treatment of rabies virus-infected cynomolgus monkeys with human interferon. (0019-9567 (Print)).

[54] Merigan Tc Fau - Baer GM, Baer Gm Fau - Winkler WG, Winkler Wg Fau - Bernard KW, Bernard Kw Fau - Gibert CG, Gibert Cg Fau - Chany C, Chany C Fau - Veronesi R, et al. Human leukocyte interferon administration to patients with symptomatic and suspected rabies. (0364-5134 (Print)).

[55] Lockhart BP, Tsiang H, Ceccaldi PE, Guillemer S. Ketamine-Mediated Inhibition of Rabies Virus Infection in vitro and in Rat Brain. Antiviral Chemistry and Chemotherapy. 1991;2(1):9-15.

[56] Weli SC, Scott CA, Ward CA, Jackson AC. Rabies virus infection of primary neuronal cultures and adult mice: failure to demonstrate evidence of excitotoxicity. Journal of virology. 2006;80(20):10270-10273.

[57] Superti F Fau - Seganti L, Seganti L Fau - Panà A, Panà A Fau - Orsi N, Orsi N. Effect of amantadine on rhabdovirus infection. (0378-6501 (Print)).

[58] Jackson AC. Is minocycline useful for therapy of acute viral encephalitis? (1872-9096 (Electronic)).

[59] Jackson AC, Scott CA, Owen J, Weli SC, Rossiter JP. Therapy with minocycline aggravates experimental rabies in mice. Journal of virology. 2007;81(12):6248-6253.

[60] Yamada K, Noguchi K, Komeno T, Furuta Y, Nishizono A. Efficacy of Favipiravir (T-705) in Rabies Postexposure Prophylaxis. The Journal of Infectious Diseases. 2016;213(8):1253-1261.

[61] Warrell M, Warrell DA, Tarantola A. The Imperative of Palliation in the Management of Rabies Encepha lomyelitis. Tropical medicine and infectious disease. 2017;2(4):52.

[62] Warrell DA. The clinical picture of rabies in man. Transactions of The Royal Society of Tropical Medicine and Hygiene. 1976;70(3):188-195.

[63] Goyal K, Garg N, Bithal P. Central fever: a challenging clinical entity in neurocritical care. J Neurocrit Care. 2020;13(1):19-31.

[64] Tarantola A, Crabol Y, Mahendra BJ, In S, Barennes H, Bourhy H, et al. Caring for patients with rabies in developing countries - the neglected importance of palliative care. (1365-3156 (Electronic)).

[65] World Health O, Food and Agriculture Organization of the United N, World Organisation for Animal H. Zero by 30: the global strategic plan to end human deaths from dog-mediated rabies by 2030. Geneva: World Health Organization; 2018 2018.

[66] Organization PAH, Center PAF-a-MD, Unit VPH. 14th Meeting of Directors of National Programs for Rabies Control in Latin America (REDIPRA). Lima, Peru; 2013.

[67] Nations FaAOotU, Control GAfR, Organization WH. The Stepwise Approach towards Rabies Elimination: A Planning and Evaluation Tool: Partners for Rabies Prevention; 2017 [Available from: https://caninerabiesblueprint.org/IMG/pdf/sare_outline_2017_f.pdf.

[68] Freuling CM, Hampson K, Selhorst T, Schröder R, Meslin FX, Mettenleiter TC, et al. The elimination of fox rabies from Europe: determinants of success and lessons for the future. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 2013;368(1623):20120142-.