Review

Eradicate Rabies with Mass Parental Vaccination, Human Post-Exposure Prophylaxis, and Gene Therapy: A Systematic Review

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Abstract: Rabies is one of the neglected tropical diseases, almost 100% fatal, but preventable. Rabies virus causes the disease and causes about 59000 human deaths annually. The author searched the Pubmed Database at NCBI for articles on rabies disease published between 2007 and 2018. All articles are open access, free for redistribution and in English. To examine rabies virus, Seller's test was used. In this article, references written by the author were included and relevant publications were also included. The author reviewed a rabies dog case kept at Nelwan Institution for Human Resource Development. The dog showed clinical signs such as aggressive behavior, in-appetence, and soaking in water. Currently, there are no drugs to treat rabies. Vaccination is the best way to prevent the disease. To eradicate rabies, mass vaccination in dogs, post-exposure prophylaxis, and gene therapy can be used. To prevent rabies disease, minimum 70% of the dog population should receive vaccination. Humans with category II exposure should receive rabies vaccine and rabies immunoglobulin. For treatment, *in vivo* experiment showed that gene therapy can eliminate rabies from the infected neurons by using rAAV-N796. To fight rabies virus, induced pluripotent cells in combination with CRISPR/Cas9 system can also be beneficial. Furthermore, it needs US$ 8.6 billion to fight rabies annually.

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1. Introduction

Rabies is one of the oldest diseases on the globe and most feared zoonotic disease known to humankind. The disease is hazardous, progressive and practically deadly encephalomyelitis [1]. *Lissavirus* is the most important rabies virus (RABV) [2]. Rabies can infect both humans and domestic animals. Most cases of rabies in animals arise among bats, carnivores, cats, raccoons, [3], mongooses, and wolves [4]. In addition, a natural rabies infection in birds has also been reported [5]. Dog (*Canis lupus familiaris*) [6, 7] is the source of more than 99% of rabies cases in humans. [8, 9].

Animal transmits RABV from animal to human through bites, or mucous membranes from saliva [2, 6] or other potentially infectious material such as neural tissue. In domestic animals, the incubation time is normally around 1-3 months. It can range from several days to months, rarely more than 6 months [2]. This incubation time in humans is usually several weeks to more than one year [3]. Non-bite sources

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of rabies are salivating, scratches [8, 10], and corneal transplantation [10]. Once symptoms of rabies begin, the disease is around 100% deadly [4, 6].

There are currently no drugs for rabies [2]. Rabies disease is 100% avoidable [9]. To prevent rabies, vaccines can be used. It needs to vaccine minimum 70% of the dog population to eradicate rabies in an endemic region. Some countries have succeeded to eradicate rabies. These countries are such as Indonesia (Bali), KwaZulu-Natal (South Africa), and Mexico. Rabies in human is avoidable through vaccination. To prevent rabies in post-exposure prophylaxis (PEP), rabies vaccine administrations and immunoglobin following contamination should be used.

Two ways are very promising for treating rabies disease. These include use of λ-Carrageenan (λ-CG) P32 and gene therapy. Luo *et al.* stated that λ-CG P32 is an anti-RABV agent that can slow down RABV infection *in vitro*. It affects viral internalization and cell fusion mediated by viral G protein. The λ-CG P32 stops viral uncoating during the viral post-absorption stage [11]. Gene therapy can include induced pluripotent stem cells (iPSCs), and CRISPR/Cas9 system. CRISPR/Cas9 is an abbreviation of clustered regularly palindromic repeats (CRISPR)/CRISPR-associated (Cas9) system. With the development of the gene therapy, the therapy of rabies treatment is also developing. To date, gene therapy has made important progress to fight rabies disease. In animal model, gene therapy can cure mice with one-day infection in the central nervous system (CNS).

To prevent rabies, billion of dollars are needed. These costs can be greater if the disease is not prevented. The evidence shows that the cost of mass vaccination plus PEP is lower than PEP only. Costs for rabies prevention may include mass vaccination, PEP, and studies. Studies may include vaccine development and gene therapy studies.

A systematic literature review is the heart of this study. This study includes rabies disease, vaccinations, gene therapy, and costs for fighting rabies. The gene therapy is for animal models only.

2. Methods

2.1. Systematic review

The present report follows the instructions of the PRISMA extension statement for systematic review [12]. These instructions also correspond to PROSPERO instructions such as searches and risk of bias assessment [13]. Below, the author quoted items from the PROSPERO written by the author.

2.2. Searches

The author searched articles for rabies in only one database; that is, Pubmed Database at National Center for Biotechnology Information (NCBI). These included free PMC articles for open access, and creative common (CC). The author only selected articles in English that published between 2007 and 2018. Keywords comprise: "canine rabies, human rabies, rabies PEP in human, rabies and vaccination, gene therapy, or iPSCs and CRISPR/Cas9 system, and costs for fighting rabies.". The author's articles regarding gene therapy were also comprised. Other articles derived from relevant publications.

2.3.Types of study to be included

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The study included a rabies case kept at the Nelwan Institution for Human Resource Development. It was a canine rabies. In this study, Seller's test was used. This study included mass vaccination, PEP, treatment and costs for fighting rabies disease. Articles included such as research articles and case study reports.

2.4. Population

The population includes people all ages.

2.5. Intervention(s), exposure(s)

1) Mass parental vaccination. All things, frequencies, and dosages were eligible for inclusion.
2) PEP. All things, frequencies, and dosages were eligible for inclusion.
3) Gene therapy. All things, frequencies and dosages were eligible for inclusion.
4) Costs for fighting rabies. Total costs, cost saving and cost effective were eligible for inclusion.

2.6. Primary outcomes

Rabies disease kills about 59000 people annually. Vaccination to the 70% of the dog population is enough to eradicate rabies disease in a region [14]. In a canine rabies-free country, this 70% requirement is not needed. However, vaccination for dogs older than 12 weeks is recommended [15].

The World Health Organization (WHO) recommends injectable inactivated vaccines for domestic dogs. Injectable inactivated vaccines in combination with bacterins and other viral antigens can be used [16]. To determine vaccine efficacy, fluorescent focus inhibition test (FFIT) and fluorescent virus neutralization (FAVN) can be used [4]. It needs at least 0.5 IU/mL of serum in both humans and dogs. WHO recommends cell culture and embryonated egg-based rabies vaccines (CCEEVs) for PEP. People with category II exposure should receive both CCEEVs and rabies immunoglobulins (RIGs) for passive protection. RIGs consist of equine rabies immunoglobulins (ERIG) and human rabies immunoglobulins (HRIG). In addition, current technology can guide to use of monoclonal antibodies (MAbs) [16].

In the future, gene therapy can be a very useful tool to treat rabies after clinical sings arise. Wu *et al.* injected AAV2-mediated RNAi (rAAV2-N796) into mice intra-cerebral. These mice were in the CNS form of the disease. The research results were 62% alive [17]. It seems that to cure rabies, gene therapy can be used.

Rabies causes around 3.7 million disability-adjusted life years (DALYs), and US$ 8.6 billion economic losses annually [15].

2.7. Data extraction (selection and coding)

The author screened titles and abstract using the search strategy to identify studies that potentially met the inclusion. Fifty-eight references for assessment were identified. The author entered extracted text in the main body of the systematic review.

2.8. Risk of bias (quality) assessment

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The author worked all items such as risk of bias assessment and data analysis. In the Results section, review results such as figure and tables were included. This is according to the PRISMA recommendation, namely, "assessment of risk of bias" [12].

2.9. Strategy for data synthesis

A narrative summary of all selected studies, and quality synthesis of the study is introduced. The author entered search results in a single figure (Figure 1). Table 1shows the total amount of human deaths due to rabies . Thirty-four references are findable in this table. Table 2 displays the percentage of total dogs receiving the rabies vaccination into. In Table 2, decrease in cases or percentage of human deaths was also included. Five references are findable in this table. The author shows recommendations for PEP into a single table (Table 3). These recommendations include the frequencies and doses of vaccination administration. Table 4 shows extraction results for gene therapy. These results consisted of the percentage of the dead mice and the administration of rAAV-N796 and rAAV-Negative. The author did not include the total amount of references in both Table 3 and Table 4. No regression analysis is findable in this study.

3. Results

The author took 163 articles from the Pubmed Database searches (Figure 1). After screening titles and abstract, 128 articles were taken for full-text review. Of these 58 articles met the criteria for data extraction. These studies were included in the main body.

3.1.A rabies case in Indonesia

Forebears of Indonesian RABVs derived from Java. The Java's RABVs offspring transmitted these rabies viruses to Kalimantan, and then to Bali, Flores, and Sumatra. The Flores's offspring transmitted these RABVs viruses to Sulawesi and went back to Kalimantan. In Indonesia, the dog was the only source of infection of other animals [18].

In this study, the dog rabies was a male, and was six years old when he died. He was the only dog in our house and never vaccinated when alive. The dog had clinical signs that include in-appetence, often urinating, thirst, heat, soaking in water, and aggressive behavior. Based on Seller's test, the dog was positive rabies. This case of rabies occurred in Palu, Central Sulawesi, Indonesia. Nelwan Institution for Human Resource Development has kept this data since Agust 6, 2015.

3.2. Rabies on the globe

More than 3.3 billion people worldwide are at risk of being infected by the rabies virus [19]. Report in the United States estimated approximately 4.5 millions dogs bite people annually [20]. The human death due to rabies is 59000 (25000 and 159000 (95 % CI: 25000-159000) people annually. Fifteen (44.12%) of 34 publications showed 59000 human deaths from rabies [8, 9, 14, 19, 26-31, 33-37]. Six (17.65%) showed 55000 human deaths from rabies annually; Table 1. Furthermore, more than 95% of human deaths due to rabies occur in Asia and Africa [9, 29, 30, 35]. Globally, around 84% of these deaths

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occur in rural areas [1]. Approximately, 50% of human deaths due to rabies are below 15 years old [10, 19, 20]. Rabies causes 3.7 million (95% CI: 1.6-10.4 million) people lost DALYs [24, 31].

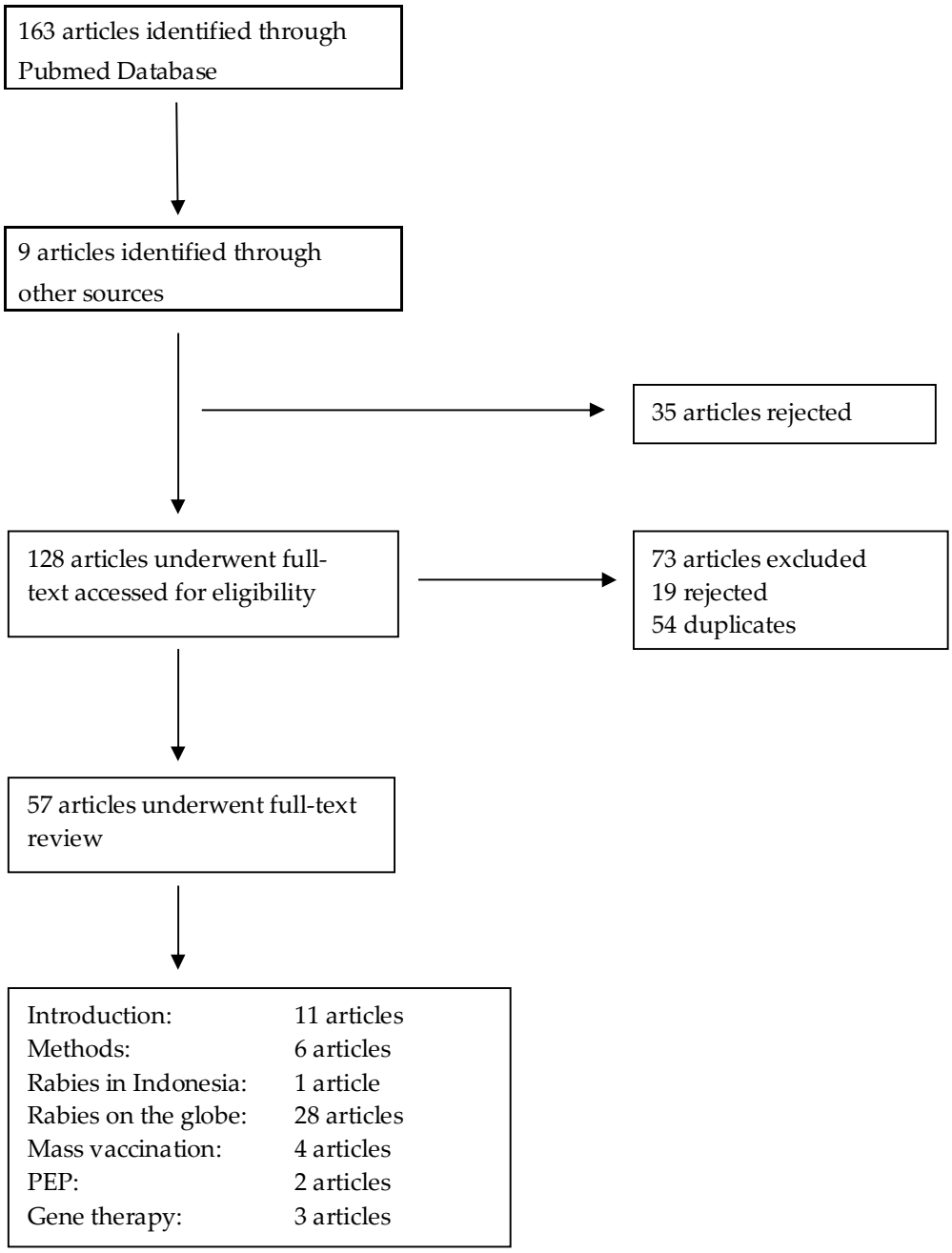

Figure 1. Flowchart showing articles selected in systematic review.

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Human deaths in Asia due to rabies exceeded 30000 annually [1, 16]. India has the highest incidence of rabies, even for all over the world. Human deaths from rabies was 16450 in India in 2010. China had 7450 human deaths in 2010 [16]. Rabies is endemic in Indonesia in 24 of the country's 34 provinces. This disease causes 150 to 300 human deaths annually [47]. In Africa, Human deaths due to rabies are about 23000 [1], 23800 [16] to 24200 [20] annually. In the Middle East and Central Asia, initial estimation can be 350 human deaths and 1900 human deaths. In Latin America, human rabies derived from dogs decreased from 250 in 1990 to fewer than 10 in 2010 [16].

Table 1. Estimation of human deaths from rabies disease every year

Human Deaths	Percentage	References	n = 34
50 000	5.88%	[21, 22]	2
55 000	17.65%	[10, 20, 23, 24, 25, 32]	6
59 000	44.12%	[8, 9, 14, 19, 26-31, 33-37]	15
60 000	14.71%	[38-42]	5
61 000	14.71%	[1, 16, 43-45]	5
70 000	2.94%	[46]	1

Human rabies in the United Stated is rare. It is only one to three cases annually. Of the 23 cases of rabies in the United States from 2008 through 2017, seven (30.43%) were rabies from dog bites. Five (21.74%) were males and two (8.69%) were females. Fifteen (65.21%) of 23 rabies cases derived from animals such as bats and foxes. One (4.3%) was unknown. Eleven (47.83%) derived from bats (contact, bite or unknown). Dog-mongoose, raccoons and fox were 4.3%, respectively. Eight (34.78%) of 23 cases were from outside of the United States and its territories [48]. Australia is free from carnivore rabies, and many Pacific Island nations have always been free from rabies and related viruses [1, 16]. Canine rabies have been eliminated in the United States [2], Canada, western Europe, Japan, and parts of Latin America countries [16]. In these areas, human deaths are restricted to people exposed while living or travelling in areas endemic for canine rabies [1, 16].

3.3.Mass parental vaccination in dogs

The WHO recommends that to eradicate rabies, at least 70% of the dog population should be vaccinated [14, 15, 16, 26, 38, 49]. It would avoid a main disease outbreak at least 95.5%, and meets the mathematical models for eradicating rabies [14]. In the endemic areas of rabies, a minimum 70% of the dog population in each year during 5-7 years should receive vaccination [15]. The crucial vaccination coverage reaches between 25-40% [43] or 30%. It is essential to interrupt rabies transmission. Mass vaccination under 30% is not beneficial for rabies eradication purposes [29]. For mass vaccination, vaccines such as Rabvac 1 and Inrab 1 can be used, for example. Both these vaccines are for dogs and cats for vaccination annually. Age at primary vaccine is 12 weeks. Route of vaccination is intramuscular or subcutaneous (Rabvac 1) and subcutaneous (Inrab 1) [3]. In a canine rabies-free country, the limit 70% threshold for eradication purposes is no longer relevant. In a canine rabies-free country, as the United

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States, most rabies vaccines are licensed for dogs older than 12 weeks of age [15]. Revaccination with a booster is one year later [15, 41].

Vaccination approaches can include door-to-door campaigns, static point campaigns, and a combination of the two [14, 16]. Such posts are usually sufficiently attended only when those posts are at less than 500 m or a 10-minute walk. The option of the approach relies on the people at local level [16].

In Asia, several countries have successfully eradicated rabies (Table 2). Sri Lanka vaccinated about 400000 in 1990 to about 1.5 million dogs in 2015. The Ministry of Health forecasts an increase in the number of rabies vaccination from present 1.8 million to 2.4 million in 2020. In this country, the incidence of rabies cases decreased from more than 50 cases in 2010 to 5 cases in 2016 [30]. In Bali (Indonesia), mass vaccination resulted in decrease of rabies cases from 72% in 2010-2011 to 90% in 2011-2012 [16].

Table 2 . New countries that have control rabies

Countries	Years	Case/percentage	References
South Korea	2014-2016	0 case	[4]
Visayas (the Philippines)	2012	13 cases	[16]
KwaZulu-Natal (South Africa)	2010-2011	0 case	[16]
Bali (Indonesia)	2011-2012	90%	[16, 18]
Sri Lanka	2016	5 cases	[30]
Tanzania	1996-2001	1 case	[50]
Argentina	2009-2017	0 case	[51]
Chile	2017	0 case	[51]
Colombia	2008-2018	0 case	[51]
Costa Rica	2017	0 case	[51]
Cuba	2009-present	o case	[51]
Elsalvador	2009-present	0 case	[51]
Ecuador	2017	0 case	[51]
Honduras	2013-present	0 case	[51]
Mexico	2006-2017	0 case	[51]
Panama	2017	0 case	[51]
Paraguay	2017	0 case	[51]
Uruguay	2017	0 case	[51]
Trinidad & Tobago	2013-2017	0 case	[51]

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In Africa, KwaZulu-Natal (South Africa) have vaccinated more than 15 million dogs since the commencement of the dog rabies eradication project in 2000. In 2012, more than 630000 dogs were vaccinated. In three years, the incidence of animal rabies has declined. KwaZulu-Natal reported in 2010-2011 a continues 12-month period without a single human case [16]. Rabies is responsible 1500 deaths annually in Tanzania. Following implementation of control activities from 2010 to 2016, human rabies deaths declined to 375 (75%) deaths [50]. Vaccination of 66% of owning dogs in Tanzania resulted in decrease in dog rabies, human PEP, and the number of positive rabies wildtype diagnosis [51].

Many countries of Latin America have successfully eradicated rabies, (Table 2). These include such as Argentina, Chile, and Mexico. These countries have no rabies case for 1 up to 10 years. Vilasco-Villa *et al*. stated that Costa Rica, Ecuador, Nicaragua, Panama, Uruguay, and Paraguay has no laboratory to assess the absence of dog maintained RABV lineages [52].

Dogs, which obtained the first vaccination after 3 months of age, would be more possible to be seropositive. Dogs obtained vaccination after 6 months of age had higher antibody titers. A recent study showed that in highly endemic areas, dogs less than 3 months can be vaccinated. It strengthens flock immunity. Vaccination more than 5-7 years results in failure rates. The optimal time to measure the immune response may be 4 -8 weeks after vaccination. The median interval is around 5 weeks for the blood sampling to measure the immune reaction [41].

For a canine rabies-free country, it is important that dogs and cats be vaccinated. In New Zealand, dogs enter the country should have been vaccinated. Vaccination should be between 6 months and 1 year before entering. Proof of a rabies antibody titer of more than 0.5 IU/mL no less than 3 months is required. In addition, This proof must be no more than 24 months before entering [15].

3.4. Post-exposure prophylaxis to human

Three categories of PEP include category I, II, and III. Category I includes touching or feeding animals, lick on undamaged skin with secretions or excretions of a rabid animal or human. It is not an exposure. In addition, it does not require PEP. Category II includes nibbling of revealed skin, small scrapes without bleeding. Finally, category III includes simple or multiple transdermal bites or scratches [16].

Category II patients should receive both CCEEVs and RIGs [16]. Currently, there are available RIGs for clinical use, namely, human HRIG and ERIG [24, 40]. Treatment after category III exposure is the immediate administration of CCEEVs and RIGs [40]. It needs to put HIRG into wound or intramuscularly for active immune response to vaccine antigen [2]. New RIG products have been available. For example, Chao *et al*. introduced SYN023 that derived from two novel MAbs CTB011 and CTB012 [53]. Um *et al*. developed 16B8-Alexa MAb evaluated using RFFIT [42]. SYN023 and 16B8-Alexa could replace the current RIG products. Both of them are safe for PEP.

Previously unvaccinated persons should receive intramuscular administration for a regime with 0.5 [16] or 1 mL [2, 16] doses of CCEEVs. For adults, the intramuscular vaccination in the deltoid area should always be administered. For children, the anterolateral aspect of the tight is also acceptable. It

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should never use CCEEVs in the gluteal area. The recommended dose of HIRG is 20 IU/kg (0.133 mL/kg) body weight for all ages of groups. If anatomically possible, the full dose of HIRG should thoroughly infiltrate in the area around and into the wound. It is a requirement to inject intramuscular any remaining volume at a site distant from vaccine administration. It requires to administer HIRG in the syringe or in the same anatomic site as the first vaccine dose. However, injector can inject subsequent doses of vaccine in the 5-dose series in the same anatomic location where the HIRG dose was administered. Rabies PEP was 100% effective in preventing a clinical case of human rabies [2].

It needs 3 ways to handle exposure. First, it handles all wounds as soon as possible, requires to wash and flush of the wounds for about 15 minutes. This needs soap or detergent of copious amounts. If possible, to wash and flush the wounds with iodine or alcohol 70% can be used. Second, it requires to administer RIG for severe category III exposures. Wounds that require suturing should be sutured loosely and only after RIG infiltration into the wound. Third, after an exposure, it should administer a series of rabies vaccine infections [54], Table 3.

Table 3. Post-exposure prophylaxis

Regime	Schedule	Days/months
Five-dose	1-1-1-1-1	0, 3, 7, 14, 28 or 0, 3, 7, 14
Four-dose	2-0-1-0-1 or 2-1-1.	0, 7, 21
High risk	1	6 months
Not continual risk	1	24 months

3.5.Treatment with gene therapy

Clinical rabies in mice can be treated. Wu *et al* constructed rAVV2 expressing siRNA targeting the nucleoprotein (N) gene of RABV (rAVV-N796). The researchers conducted a study with two treatments on mice. The study included administration of rAAV-N796 (intracerebral or intramuscular), or rAAV-Neg (intracerebral or intramuscular). In other treatments, the mice received 10 LD50 or 20 LD50 of deadly CVS. The highest result was obtainable in intramuscular rAAV-N796 treatment and intramuscular treatment of 20 LD50 (C). The result was 62% on day 21 after infection [17]; Table 4.

The author's did not find any reference relating to iPSCs and CRISPR/Cas9 system for treating rabies. However, Nelwan indicated that gene delivery tools, gene-editing tools, and iPSCs are useful to treat monogenic disorders in animal models [55-58]. Therefore, gene therapy can be useful to treat rabies disease.

3.6. Estimated burden of rabies in the world

The annual cost for rabies prevention varies from region to region, including one country to another. Asia needs as much as US$1.5 billion for PEP only. European Union and Pan American spend

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US$6.5 million and US$20 million, respectively [16]. The United States needs US$ 300 million annually. [1, 16]. To control fox rabies during the period of 1986-1995, France spent approximately US$ 261 million [16].

The estimated annual cost of rabies is US$ 8.6 billion worldwide [14, 30, 45]. Latin America needs US$129 million for PEP and needs US$61 million for mass vaccination. It is the most cost effective restrictive approaches [26]. PEP needs US$1.7 billion annually [26, 50] and 3.7 million DALYs annually [14, 30, 50]. The cost of canine rabies vaccination was US$130 million in the endemic countries of Asia and Africa [50].

Table 4. Treatment with rAVV-N796, rAAV-Neg, 10 LD50, 20 LD50

Days post infection				
	rAVV-N796	**rAVV-Neg**	**Survival rate**	
Treatment	Intracerebral		rAAV-N796	rAAV-Neg
Intracerebral	9	9	100%	100%
10 LD50 (A)	21	21	45%	0%
Treatment	Intramuscular		rAAV-N796	rAAV-Neg
Intramuscularly	9	9	100%	100%
20 LD50 (B)	21	21	38%	0%
Treatment	Intracerebral		rAAV-N796	rAAV-Neg
Intramuscular	9	9	100%	100%
10 LD50 (C)	21	21	62%	0%
Treatment	Intracerebral		rAAV-N796	rAAV-Neg
Intramuscular	9	9	100%	100%
20 LD50	21	21	62%	0%
Treatment	Intramuscular		rAAV-N796	rAAV-Neg
Intramuscular	9	9	100%	100%
20 LD50	21	21	20%	0%

4. Discussion

Humans have been living in fear of rabies outbreaks of dogs since thousands of years ago. Currently, more than half the world's population is still fearful of rabies outbreaks. The first report in rabies has been around since 2300 BC [26]. Rabies has already existed in Indonesia since 1884 [47]. This disease is endemic in provinces such as Central Sulawesi, North Sulawesi, and West Java. Rahmadane *et al.* stated that Indonesian rabies belong to the Asian lineage; that is, *lyssavirus* genotype 1 [47]. Indonesia regularly control rabies disease at provincial, district [47], and municipal levels. However, sufficient

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vaccination coverage has been hard to reach [47]. Indonesia and other ASEAN countries expect free of rabies in 2020.

Canine rabies clinical signs can include aggression, abnormal behavior, vocalization changes, paralysis [3, 16], ataxia, cranial nerve deficits, dysphagia, in-appetence [3], drooling, and convulsions [16]. The dog in this study had clinical signs such as often urinating and soaking in water. However, he had an in-appetence and aggressive clinical signs. The dog did not have clinical signs such as ataxia and paralysis. Other clinical signs he had were thirst and heat. It seems that dog clinical signs are not the same as described in reference 3 and reference 16, except in-appetence and aggressive behavior. It seems that often urination, soaking in water, thirst and heat are new clinical signs of canine rabies.

The Seller's test is a rapid and a simple method. Limitation of this test is only suitable for fresh samples. In addition, the Seller's test has a very low sensitivity [1, 47]. Indonesia uses Seller's test, fluorescent antibody test (FAT), and mouse inoculation test (MIT) to detect rabies virus. If the Seller's test is negative, the laboratory sends it to the FAT laboratory. If still negative, the laboratory sends it to the MIT laboratory [47]. Fortunately, the dog in this study had positive rabies based on the Seller's test. Therefore, it did not need to use FAT or MIT to determine the rabies status.

Mass vaccination in dogs and the administration of PEP are important items to eradicate rabies disease. Mass vaccination should meet the minimum 70% of the dog population. PEP in humans should be handled immediately. Failure to handle victims, it can be fatal for them. Salomäo *et al.* showed that dogs are the only animal that bit humans in Maputo and Matola cities (Mozambique). This was an expected discovery and confirm data from other countries. It suggests that rabies eradication efforts should focus on dogs [20].

The PEP is a human condition that has had dog bites. Both dogs and humans who get dog bites should receive serious observation. The biting dog needs to receive observation for 10 days. If the dog remains healthy, it does not need to continue vaccination. If the dog dies within 10 days, it needs to continue vaccination. Vaccinations consist of either a five-dose schedule (1-1-1-1-1) or a four-dose schedule (2-0-1-0-1 or 2-1-1); Table 3. For passive protection it needs only once vaccination.

Currently, there are no effective drugs for rabies disease. Medical treatments currently focus on mass vaccination and PEP for instance. However, gene therapy may be a very useful tool to treat rabies in the future. This technique can include gene delivery vectors such rAAV [55] and CRISPR/Cas9 system [56], and iPSCs technique. The iPSCs technique is helpful for disease modeling, drug screening, and stem cell therapy [57]. This technique in combination with a CRISPR/Cas9 system for treating rabies may be developed. It means drugs derived from this combination may be useful to treat rabies in wildtype animals for instance.

Lavan *et al*. showed the cost benefit for mass vaccination and human PEP and human PEP only. The costs were based on the annual cost of the rabies project in Bhutan as long as 6 years. Each stage consisted of 2 years. Vaccination coverage was 70% in stage 1, 60% in stage 2, and 50% in stage 3. The number of PEP cases annually was 3440. During the stage 1, the costs of mass vaccination and human PEP exceeded the costs of human PEP only. During the stage 2, costs of mass vaccination and PEP was less than costs of human PEP only. At the stage 3, costs of mass vaccination and human PEP were lower

than costs of human PEP only. It was US$ 730,000 against US$ 770,000. It shows a cost saving and cost effective intercession [50].

5. Conclusions

Rabies is a neglected tropical zoonotic disease and is an urgent disease. The disease is nearly 100% deadly and 100% avoidable. Clinical signs of rabies disease can include such as often urinating, inappetence, and soaking in water. Vaccines are the only way to fight the rabies virus at present. In the future, to fight rabies, gene therapy, iPSCs technology, and gene editing tools can be very valuable. The iPSCs technique in combination with CRISPR/Cas9 system can be useful to eradicate this disease. It shows that mass vaccination, PEP, and gene therapy can help to eradicate rabies disease worldwide. Costs to fight rabies with mass vaccination and PEP is lower than costs of human PEP only.

List of abbreviations: RABV: Rabies virus; PEP: post-exposure prophylaxis; λ-CG: λ-Carrageenan; iPSCs: induced pluripotent stem cells; CNS: Central nervous system; CRISPR: clustered regularly palindromic repeats; Cas9: CRISPR associated 9; NCBI: National Center for Biotechnology Information; CC: creative common; WHO: World Health Organization; FFIT: fluorescent focus inhibition test; FAVN: fluorescent virus neutralizalization; CCEEVs: cell culture and embryonated egg-based rabies vaccines; RIGs: rabies immunoglobulins; EIRG: equine rabies immunoglobulin; HIRG: human rabies immunoglobulin; MAbs: monoclonal antibodies; DALYs: disability-adjusted life years.

Conflicts of interests: The author declares that there are no competing interests.

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Author's contribution: MLN conceived and design of the study, participated in its design, draft the manuscript, and involved in revising the manuscript critically for significant intellectual need. The author read and approved the final manuscript.

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