Review Article

One-Hundred-Year Efforts by the Pasteur Institute of Iran as a Part of the Rabies Infectious Control Puzzle in the World

Maryam Fazeli[1], Ahmad Fayaz[2], Rouzbeh Bashar[1*]

[1] The National Center for Reference and Research on Rabies, Virology Department, Pasteur Institute of Iran, Tehran, Iran
[2] Scientific Advisor of Pasteur Institute of Iran, Tehran, Iran

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Abstract

Rabies is a fatal zoonotic disease that occurs usually via animal bites. Rabies is currently an economic health problem in Iran and almost all of the provinces are more or less involved in this. For nearly a century, many researchers have tried to control rabies in Iran. To date, Iran has played a major role in the new scientific advances in the rabies treatment field. The Pasteur Institute of Iran was established in a 2.2 ha land to provide public health services for the prevention and treatment of infectious diseases. Since then, the official activity of the Pasteur Institute of Iran has begun. The operations of the Institute are still being developed quantitatively and qualitatively. The aim of this study is to introduce One-Hundred-Year Efforts by the Pasteur Institute of Iran as a Part of the Rabies Infectious Control Puzzle in the World.

Keywords: Rabies disease, Infectious disease Control, Iran

***Corresponding Author:** Rouzbeh Bashar, The National Center for Reference and Research on Rabies, Pasteur Institute of Iran, 12 Farvardin St., Tehran, Iran. Tel: (+98) 21 66403496, Fax: (+98) 21 64112813, E-mail: bashar@pasteur.ac.ir

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1. Introduction

1.1. Pasteur Institute of Iran: The Pasteur Institute was established on June 4, 1887, by Louis Pasteur (1822–1895) as an anti-rabies center in Paris and officially launched on November 14, 1888[1, 2]. After a short time, the center was overcrowded, so that the facility could not respond to all patients. As soon as the Pasteur Institute was established, it attracted scientists with various professions[2].

In 1919, one year after the World War I (WWI), the Iranian government of that time tried to revive its scientific relations with France to enhance the status of medical science and research on various types of endemic infectious diseases in the country despite the fact that it was still suffering from fatalities, famines, and illnesses as well as the hardships resulting from the war[3]. So, Iranian political delegation traveled to Paris for a peace conference to reach this goal. They met Professor Emile Roux (Figure 1), the director of the Pasteur Institute of Paris in that time, in October 1919[3]. The meeting was the starting point of the establishment of the Pasteur Institute of Iran and Joseph Ménard (Figure 1) was nominated for the director of the Pasteur Institute of Iran. About three years after the meeting on January 20, 1921, professor René Legroux signed the agreement on behalf of Pasteur Institute of Paris with the Iranian Foreign Minister, which served as a model for the technical cooperation between the two countries[3]. So, the

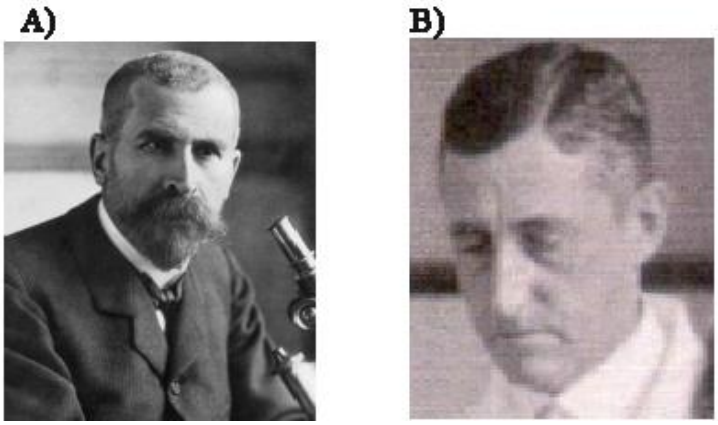

Figure 1. (A) Dr. Emile Roux (1853–1933), the director of the Pasteur Institute of Paris at the time of the Pasteur Institute of Iran establishment; (B) Dr. Joseph Ménard, the first French director of the Pasteur Institute of Iran.

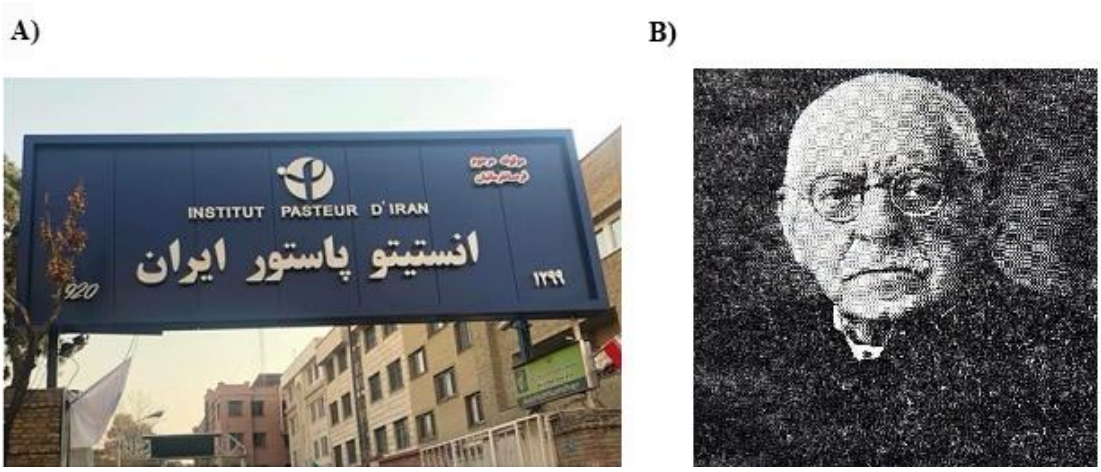

Figure 2. (A) Pasteur Institute of Iran entrance, Tehran, Iran; (B) Mr. Abdol-Hossein Farman Farma (1852-1939).

Pasteur Institute of Iran was established in a 2.2 ha land donated by Mr. Abdol-Hossein Farman Farma (Figure 2) to provide public health services for the prevention and treatment of infectious diseases. Since then, the official activity of the Pasteur Institute of Iran has begun. The operations of the Institute are still being developed quantitatively and qualitatively[3].

2. The History

Rabies is a fatal zoonotic disease and an important public health problem in the world. The disease is caused by neurotropic viruses in the genus Lyssavirus, family Rhabdoviridae (ICTV 2015)[4]. It is primarily transmitted to humans via the saliva of infected animals after biting. Scratching or licking of mucous or wounds by rabid animals is other routes that rabies can be transmitted[5]. Initially, it begins to replicate in the muscle at the wound site. After reaching the central nervous system of the host, the virus developed progressive encephalopathy with a fatal outcome[5]. Currently, rabies is considered as a neglected and emerging disease in low-income and developing countries[6].

Rabies has a long history, most of which have been lost in the distant past. Akteon, a famous mythical hunter, was torn to pieces by his dogs and his corpse was found by his entourage in the bathroom. Akteon seemed to be killed by a rabid dog[7]. The term of rabies is derived from the Sanskrit term rabhas. The disease was historically characterized by the anger of the gods, the spell of the witches or extraterrestrial attacks. The term “lisa” has been translated in English as bitten by a dog[8]. In the Dehkhoda Dictionary (Persian), rabies

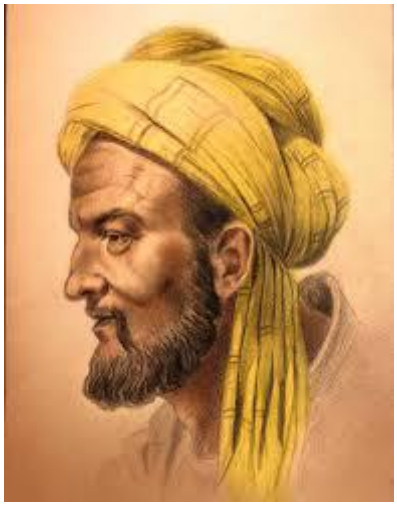

Figure 3. Avicenna or Ibn Sina (c.980-1037) Persian polymath who's encyclopaedia of medicine 'The Canon of Medicine' was the authoritative work on medicine throughout medieval times.

has been interpreted as a crazy animal, especially crazy dog.

In Iran, rabies was known to peasants and shepherds from ancient times. They knew that the disease is transmitted to humans by rabid dogs and, sometimes, by wolves[9]. Ibn Sina (980–1037 AD) (Figure 3), the Iranian well-known scientist, introduced the disease as the fear of water (hydrophobia) and recommended that the bitten site is not covered for 40 days and blistering materials are used in the wound. He has explicitly stated that the blood from the rabid dog is an antibody of its bite. This treatment method, along with burning the wounds was the major treatments of animal bites up to the early twentieth century[9]. In 1885, Pasteur introduced the first weakened vaccine of rabies [10]. Pasteur suspended the spinal cord of the infected rabbits in potassium-containing plate. He immunized some dogs by the intradermal injection of the suspension containing the small piece of the spinal cord[10]. The internal cytoplasmic features when discovered by an Italian scientist, Adelchi Negri (1876–1912), in 1903, became important in the laboratory diagnosis of rabies[11].

Up until the establishment of the rabies center in the Pasteur Institute of Iran, persons who were bitten by dogs, resorted to superstitious for the treatment and sometimes even lost their lives due to these activities. In 1923, two years after the establishment of the Pasteur Institute in Iran, a foreign ambassador's son in Tehran was injured by a suspected rabid dog and taken to the Pasteur Institute for treatment, while the institute still lacked anti-rabies services. They were forced to take the child out of Iran for treatment. This led to the decision of the authorities of the Pasteur Institute of Iran to establish an anti-rabies service and the service was launched in the institute in 1923 under the supervision of Dr. Mehdi Ghodsi (1900-2000). Gradually, other centers were established in other provinces and cities. According to geographic distributions, different animals could be a source of the disease. In Iran, rabies is more common in the fall and winter, in men than women and under 20-year-old subjects[12].

To date, Iran has played a major role in the new scientific advances in the rabies treatment field. For example, in 1954, Professor Marcel Baltazard (1908–1971), the director of the Pasteur Institute of Iran and his assistant Dr. Ghodsi, concluded that anti-rabies treatment was totally valid in its used form, but not sufficiently effective[13]. The use of the vaccine obtained from the rabbit spinal cord continued until 1936, and then, Dr. Ghodsi developed the anti-rabies vaccine by the Fermi method using the rabbit's brain. Fermi vaccine was used in Iran for ten years[14]. Dr. Sabeti, the head of the vaccination center, who was in charge of treating the rabies-infected individuals for some 30 years, believed that due to the problems of breeding the rabbits, the Fermi anti-rabies vaccine could cause a nerve reaction called rabbit blow[15]. Therefore, in 1946, Dr. Ghodsi prepared the Semple

rabies vaccine. He observed the behavior of rabies-infected subjects for years and followed closely the treatment results. In his 10-year observations, he found that the classic Pasteur treatment is not sufficient and effective in those with severe wounds due to the wolves' bites and that some of those who were treated lost their lives due to rabies[16]. He published two shocking articles, which became a milestone in the history of anti-rabies treatment. The first article was published in 1947 in the journal of the Pasteur Institute of Paris and the second in 1955 in the World Health Organization bulletin. In the two articles, Dr. Ghodsi evidenced the inadequacy of the anti-rabies vaccine when used alone for the treatment of rabies-infected individuals with deep and numerous wounds on the head and face[14]. Publication of these articles by the Iranian scientist made the authorities of the World Health Organization (WHO) aware of the defects in the treatment of the rabies-infected individuals. Consequently, the WHO Experts Committee on rabies decided to provide the Pasteur Institute of Iran not only with the rabies vaccine but also with the anti-rabies serum obtained by immunizing a number of rabbits for the treatment of subjects who were bitten by wolves[14]. The Iranian scientists at the Pasteur Institute sought the opportunity to treat those who were bitten by wolves with rabies vaccine and serum simultaneously. They waited four years for such an opportunity. In 1954, a wolf attacked residents in the Sahne county in the Kermanshah Province, wounding 29 people. The wolf was killed and the rabies virus was found in its brain and saliva. The injured subjects were transferred to the Pasteur Institute of Iran in Tehran for treatment. Director of the Pasteur Institute of Iran, Dr. Marcel Baltazar with Dr. Mahmoud Bahmaniyar (1919-2007) immediately used the anti-rabies vaccine and serum to save the injured subjects in two groups with the following methodology; the first group consisted of eighteen subjects with numerous and deep wounds on their head and face, 13 among whom received one or two rabies serums and a set of vaccination, and five only received the anti-rabies vaccine[14, 15]. The second group included eleven injured subjects with weak wounds away from their head and face, who were divided into two groups. The first group was treated with the serum and vaccination and the second only with the vaccine. The result was very significant and successful. From among thirteen patients treated with a combination of serum and vaccine, despite their deep wounds on the head and face, only one caught rabies and was died and from among the five subjects who were treated with the vaccine, three died. Among the eleven subjects of the second group who had mild injuries, no one caught rabies. The results of the treatment of the injured subjects were reported to the WHO Expert Committee in Geneva[15]. Subsequently, the Expert Committee suggested in 1955 that the combined anti-rabies serum and vaccine treatment method is the best way to save the rabies-infected subjects worldwide[15]. This method is used to treat rabies all over the world.

In Iran, the anti-rabies serum of Heterologous was being produced for years by Dr. Hossein Mirshamsi (1917-2008) and collogues at the Razi Institute. The serum was highly-graded and supplied in the lyophilized form; however, its consumption was halted due to its allergic complications. Instead, the use of the anti-rabies serum produced in humans or anti-rabies immunoglobulin was made common[17].

The problem remaining in the treatment of rabies was complications of the vaccine produced from nerve tissues which occasionally led to paralysis after the treatment. The same problem was also observed in other countries of the world and was known as the postvaccinal paralysis. Robert Kissling (1923-2013), the Centers for Disease Control and Prevention (CDC), Atlanta, showed that the rabies virus could be proliferated on non-nervous cells in the in vitro environment for the first time[18]. In 1964, researchers from the Wistar Institute in Philadelphia successfully proliferated the rabies virus on the human diploid cell and prepared the empirical human diploid cell rabies vaccine (HDCV) vaccine. They proved the immunogenicity power and harmlessness of the vaccine by injecting it to 16 of their colleagues[19]. In 1975, the vaccine was built on a large scale in France and the manufacturers successfully obtained the license for its use in human beings. It was then applied to immunize the students of the Faculty of Veterinary Medicine in France[20].

In 1976, the Pasteur Institute of Iran was assigned by the WHO to evaluate the HDCV vaccine. The Iranian

researchers first used this vaccine to immunize five volunteer groups by various methods. After evaluating the antibody in the volunteers, they considered the method of injecting the vaccine on days 0, 3, 7, 14, and 30 and the injection of a booster dose on day 90 as the best prevention treatment method. Researchers at the Pasteur Institute of Iran used the method to treat 45 subjects who were severely injured by two rabid wolves and six rabid dogs. All of these subjects were rescued from the risk of rabies[21]. At the same time, the results of their treatment, in addition to the results of the treatment of another group of rabies-infected subjects in the city of Essen, Germany, were examined by the WHO Expert Committee on rabies and was popularized around the world as the Essen method according to the recommendations of WHO[22].

In 1980, the researchers of the Pasteur Institute of Iran examined the immunity status of 45 patients treated in 1976. They took blood samples from 27 of the subjects before and after injecting a single dose of the follow up vaccine. It was determined that the mean antibody titer of these individuals after four years of treatment with the HDCV vaccine was 20.6 IU/Serum, and after injecting a vaccine booster dose, the average titer reach 288 IU/Serum. The results showed that in people treated with HDCV vaccine, in case the individual is bitten again after four years, injection of a dose of a follow up vaccine would prevent rabies[23,24].

32 years after the treatment, the researchers at the Pasteur Institute of Iran decided to reinvestigate the 45 rabies-infected subjects treated with HDCV vaccine in 1976 in terms of the strength of the rabies antibody inhibitor. Therefore, the researchers went to villages and cities where the 45 subjects were living 32 years ago, and they could find 26 of them. The rabies antibody was still present in all of the subjects after 32 years, and when two follow up doses of the vaccine were injected in 3-day intervals, the antibody level was significantly increased. This indicated that those who have been treated with the HDCV vaccine once after animal bites could be treated with two doses of vaccine in a 3-day interval to prevent rabies if they are bitten again after 32 years[24].

During the past 40 years, due to the efforts of the Pasteur Institute of Iran and the Center for Reference and Research on rabies, Treatment and Prevention of rabies have undergone a number of changes to save lives of those bitten by rabid animals in the country. Problems caused by the injection of heterologous serum have been resolved. The neurological complications of brain tissues, which in some cases caused fatalities due to paralysis, are no longer observed due to the use of cell vaccines. The number of doses from 14 to 4 times in Zagreb regimen declined. Most importantly, the fatality rate of the treated patients has decreased significantly[14]. For example, in 1971, 1243 individuals bitten by rabid animals were treated and 13 were dead due to rabies. In 2010, however, more than 120000 bitten subjects received prevention-therapeutic treatments for rabies and the fatality rate was only 5 subjects[25].

3. The Situation of Animal Bite and Rabies in the World

The virus occurs across all parts of the world except Antarctica (Figure 4)[25]. Approximately 20,000 human cases, which account for about one-third of annual rabies cases, occur in India, mostly through canine bites. Thus, in this country, the primary control measure has focused on the elimination of rabies in animals, mainly dogs[27]. People of any age are susceptible to rabies, but children are more at risk for the disease[28]. Although rabies is endemic in the animal populations in many European countries, most cases of rabies have been observed in Asian and African countries and dogs are the main carriers of the disease in these countries[29].

According to the investigations, in 2019, 89.7 million dogs and 94.2 million cats were being kept as pets in the US. In fact, one-third of the US population had pets. In this country, about 4700000 cases of animal biting are recorded each year, but due to the vaccination of pets and adequate educations, only about 30,000 to 50,000 cases of post-exposure treatment are performed[30].

In a study performed in Turkey, during 1990-2000, 2,856 rabies cases were reported and 78% of these cases were reported from dogs and only 1.6% was from wildlife[31]. Most of the reported rabies cases during 2000 – 2010 in Azerbaijan were found in dogs, cattle, and sheep[31].

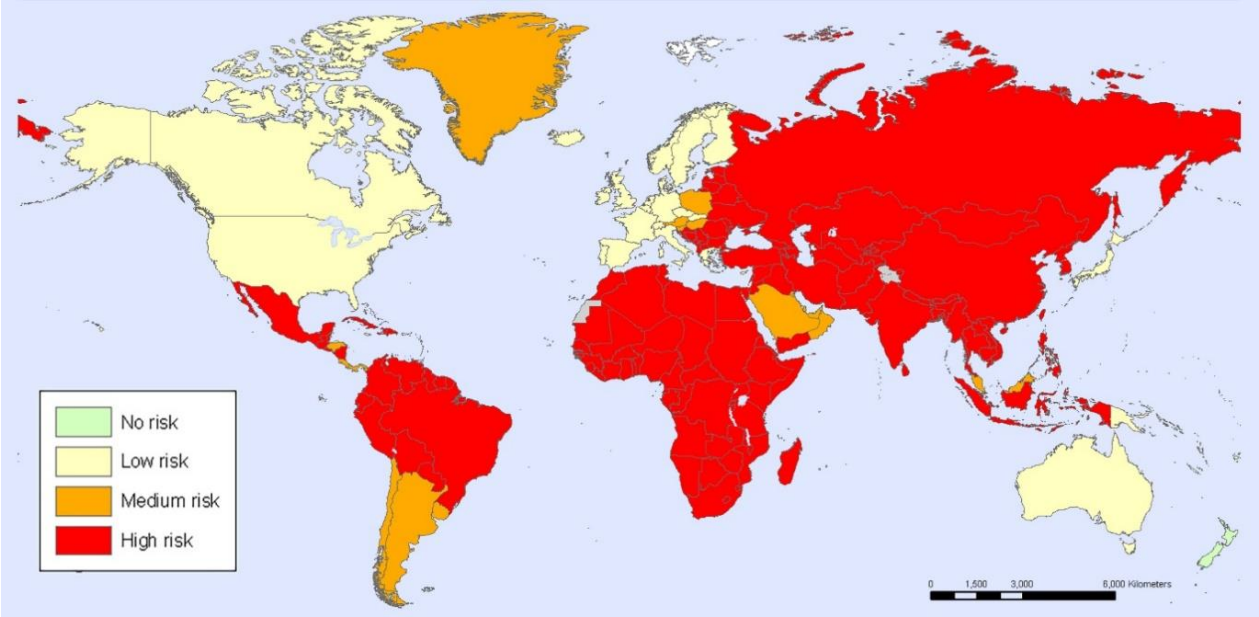

Figure 4. Global distribution of rabies (WHO, 2013). No risk: there is no risk virus transmission; Low risk: pre-exposure immunization recommended for people who might come into contact with bats; Medium risk: pre-exposure immunization recommended for travelers and other people who might come into contact with bats and other wildlife; High risk: pre-exposure immunization recommended for travelers and other people who might come into contact with domestic animals particularly dogs and other rabies vectors[31].

4. The Situation of Animal Bite and Rabies in Iran

The pet keeping trend is increasing in Iran as with other countries in the Middle East and Asia. About 180000 cases of biting are observed each year in Iran; all of whom receive post-exposure treatments due to the suspicions of the pets and inadequate educations. Of these, about 81% of subjects are bitten by dogs and 69.4% of dog bites are related to the owned dogs[32] (Figure 5).

Rabies is currently an economic health problem in Iran and almost all of the provinces are more or less involved in this. The most cases of the disease are observed on the edge of the Caspian Sea, northeast and southwest of the country. According to the Ministry of Health and Medical Education, an annual of 160,000 individuals are treated by the Pre-exposure prophylaxis (PrEP) measurement[33]. According to the ante-mortem diagnosis of rabies data of the National Center for Reference and Research on Rabies, the Pasteur Institute of Iran, 5-11 individuals die from rabies in Iran annually. Considering that the disease is spread throughout the country, preventive measures must be taken in all provinces (Figure 6).

5. The National Center for Reference and Research on Rabies, Pasteur Institute of Iran

The center after establishing in 1923 was selected as the collaborating center by the WHO from 1973 due to its intensive international cooperation in the field of rabies. The collaboration position is renewed every four years, according to the terms of reference submitted by the department to the EMRO (Eastern Mediterranean Office) and by completing the WHO collaborating centers questionnaires. Since 2014, the center has obtained the national rabies authority certificate after implementation of ISO 15189 and as a collaborating center for the health reference laboratory of the Iran Ministry of Health and Medical Education.

The establishment of ante-mortem rabies diagnostic laboratories in the country is also among the activities of this department. Missions of the department include the maintenance of scientific and technical achievements, the enhancement of quality and planning for sustainable development in the country

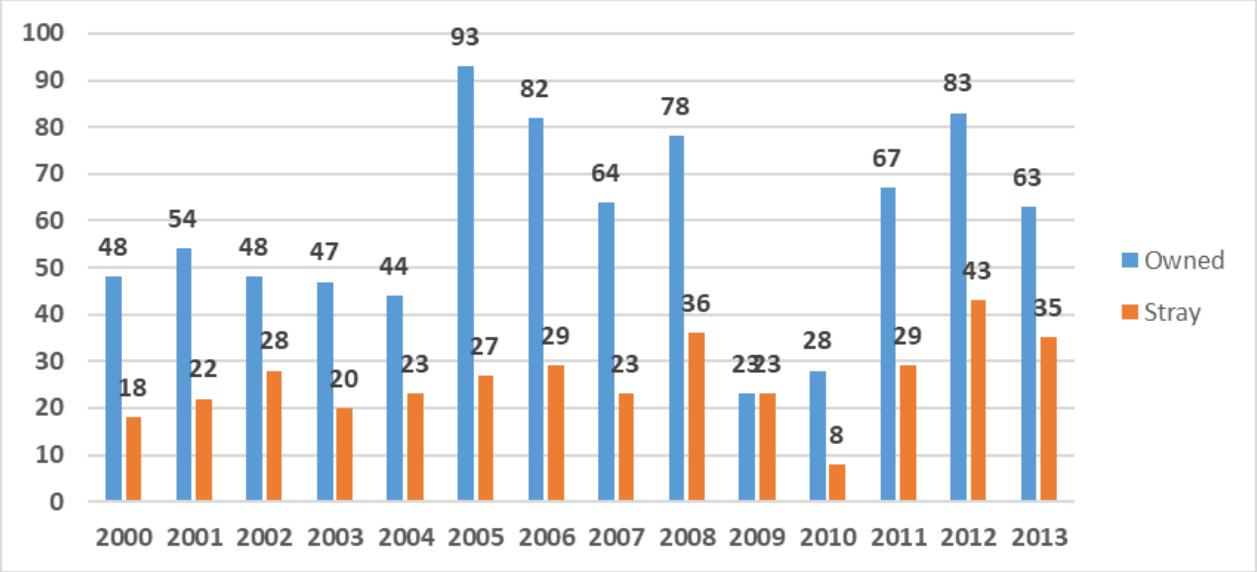

Figure 5. Comparison of the distribution of rabies in owned and stray dogs in Iran, 2000-2013 (n=1,186)[30].

and the region. In this regard, the quantitative and qualitative enhancement of the bilateral, or multilateral, educational, research, and technical cooperation of the center with relevant and international public agencies for controlling rabies and aiming at its elimination are long-term missions of the department.

The department is divided into five laboratories:

5.1. Routine Rabies Diagnostic Laboratory: In the laboratory, the rabies-suspected brain samples of animals or human are investigated by the accurate and sensitive laboratory methods such as FAT and MIT and the results are sent to the center requesting the test in a daily manner. In this unit, the annual rabies statistics, including the total number of subjects bitten by rabid animals, rabies-related fatalities, the prevalence of rabies in human and animals in different parts of the country are discussed and analyzed. The Chief Executive Officer (CEO) of the laboratory prepares updated GIS maps on the prevalence of rabies.

5.2. Serological Laboratory (Rapid Fluorescent Focus Inhibition Test (RFFIT)): In this laboratory, the anti-rabies-specific antibody assay is performed by the RFFIT method on human or animal sera. Controlling of the antibody titers in imported immunoglobulin sera as well as the safe domestic sera are performed by the experts in this laboratory. In addition, the Ab-rabies titer is measured by the ELISA method, which is a quicker method than the RFFIT and is used for screening. The antiviral effects of some medicines, as well as the virucidal effect of some imported or domestic disinfectant substances, are also examined in this laboratory.

5.3. Laboratory for evaluating vaccines and biological products: In this laboratory, the potency of imported and domestic rabies vaccines for human and animal consumption is evaluated by National Institutes of Health (NIH) method (recommended by the WHO). After confirming the immunization level of the tested vaccines in this laboratory, based on the capabilities and experience of the rabies reference center and due to its collaboration with other relevant governmental organizations, such as Ministry of Health and Veterinary Organization, could allow the marketing of the vaccine in question. In the research and development (R&D) unit of the laboratory, research projects on the rabies vaccine and their immunization effect are being performed.

5.4. Molecular laboratory to the diagnosis of rabies: In this laboratory, molecular studies and diagnosis on the genome of isolated strains obtained from all over the country are performed such as RT-PCR, Real-Time PCR and RT-LAMP. Other examinations performed in the department include the molecular analysis of infected brain, cerebrospinal fluid, skin, saliva, urine, and sera samples from ante-mortem and post-mortem rabies-suspected subjects.

5.5. Molecular epidemiology of rabies: In this unit, phylogenies and phylodynamic studies are conducted to monitor the distribution of wild rabies strains in different parts of Iran. According to the latest publication by Dr. Nadin-Davis in 2003 and by sequencing of a part of the virus genome (350 bp of a phosphoprotein gene), it cleared that the major variant of the rabies virus circulating in Iran is cosmopolitan lineage along with a few Arctic variants[34]. In a recent cooperative study by the Pasteur Institute of Iran and Paris in 2017, 150 samples were selected from different geographic regions and sequencing was performed for the total length of the viral genome. Preliminary results of this study indicate that circulation variant has not changed in comparison with Nadin-Davis study. Iran's Cosmopolitan variants are categorized into four different groups of Middle Eastern variants and Central Asian variants.

6. Measures Taken so far in the National Center for Reference and Research on Rabies, Pasteur Institute of Iran

6.1. Member of the National Committee of rabies in the Iran's Ministry of Health and Medical Education (MOH): Member duty is evaluation and selects an anti-rabies serum and vaccine purchasing sources required for more than 700 rabies prevention-treatment centers. In addition, revisions and updates on the most recent therapeutic protocols used in this regard (according to the invitation of the national rabies committee of the MOH). The director of the center along with his experienced experts attends in the meetings and provides the necessary guideline on questions raised.

6.2. Collaboration on drafting the National Vaccine Program and the use of anti-rabies safe serum at the MOH: Considering the annually increasing consumption of the biological agents in the prevention of rabies and according to the proposal of the center for the disease control of MOH, the center implements projects in cooperation with the MOH to mediate the optimum consumption of rabies serum and vaccine. The consumer of the biological agents is more than 180,000 bitten subjects annually. After analysis, the results of the projects will be proposed in national operations in the National Committee on Rabies.

6.3. Collaboration on revising programs, protocols and identifying strategies for controlling and eliminating rabies in the country: The center has a strategic and central role in various meetings held with other organizations involved in this field (veterinary organization, environmental protection organization, rural and urban municipalities and ministry of health). It analyzes the experiences obtained by other Asian and European countries along with the experts from the French, Sri Lanka, and Pakistan to try to formulate a native plan for the control and elimination of rabies in the country.

6.4. Collaboration on the implementation of educational preventive and treatment programs for rabies of communicable diseases Center, Iran's MOH: According to the formulated educational programs implemented by the CDC of Iran, the research and reference center of rabies has actively participated in all educational programs in Tehran (Pasteur Institute of Iran) and other provinces of the country, explains the latest therapeutic and diagnosis methods for the experts in health centers or veterinary experts.

7. The National Center for Reference and Research on Rabies, Pasteur Institute of Iran and International Centers

Pasteur Institute is currently one of the world's leading research centers, with 100 research units with nearly 2,700 employees, including fixed 500 researchers and 600 contractual researchers, who visit some 70 countries of the world annually. The institute also includes a network of 28 centers for addressing medical problems, specifically in the developing countries, all of which have a Pasteur Institute of their own. These 28 centers include (all in the capital of the country, unless another city is specified): Algeria, the Central African Republic, Belgium, Brazil (Sao Paulo), Cambodia, Senegal, France (Lille), Guadeloupe, French Guiana, Vietnam, Iran, Ivory

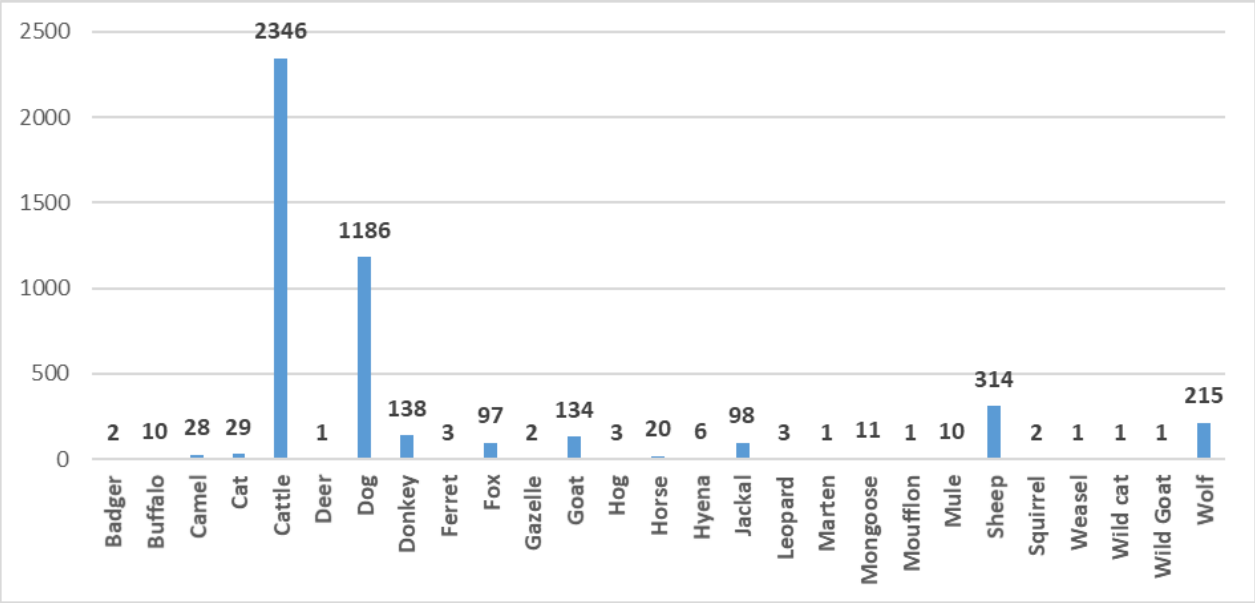

Figure 6. The species distribution of confirmed cases of animal rabies in Iran 2000-2013 (n=4,663)[30].

Coast, Madagascar, Morocco (Casablanca), New Caledonia, Russia (St Petersburg), Tunisia, Greece, Uruguay, Romania, Niger, Cameroon, South Korea, China (Shanghai), USA (New York), Canada (Montreal), Hong Kong and India (Coonoor).

7.1. Collaboration with WHO: The center collaborates with WHO in different ways, including:

1. Collecting statistical data on rabies including the number of subjects who bitten, the number of subjects who treated for rabies, the number of treatment cases using the vaccine alone or the vaccine plus serum, the type of biting animals. These types of data should also be prepared and submitted on the animal rabies.
2. Participation in rabies control programs as a counselor in different counties of the region, including Pakistan, Afghanistan, and Yemen.
3. Provision of the EMRO/WHO countries with the required guidance to control rabies.
4. Research collaboration, especially on issues raised by the experts' committee, scientific groups, and advisory meetings.
5. Educating the health personnel on the principles of anti-rabies treatment in the countries of the region.
6. Participation in the preparation and evaluation of reference products.
7. Preparation and submission of standard strains of rabies virus to other laboratories.
8. Diagnosis of suspected rabies virus variants and evaluation of vaccines and serums from other laboratories.
9. Providing advisory services on controlling rabies and anti-rabies treatment at the request of the WHO.

7.2. Establishing communication bridges between organizations involved in controlling, treatment and elimination of rabies with relevant international centers, such as participate in the meetings of rabies expert in Geneva, participating in periodic meetings of Middle East, Eastern Europe, Central Asia and North Africa Rabies Expert Bureau (MEEREB): By the annual invitation of the WHO general assembly of experts in rabies in Geneva, the head of the department actively participates in these assemblies. He presents challenges, advances and other aspects of rabies control in developing countries, not only to advance the process of the assigned mission, but also to communicate information and manners with other centers collaborating with the WHO. In addition, providing this information for the relevant bodies such as the Veterinary Organization, the Environmental Protection Organization.

8. Measures of the Pasteur Institute of Iran in Relation to Epidemiology and Control of Rabies in Iran

In 1981, there was a record about 59 animal rabies cases that were confirmed by the Pasteur Institute of Iran[12]. In this year, a project was implemented in Tehran (the capital city of Iran) under the name of controlling rabies in stray dogs. In 1988, Tehran was free of rabies and the situation continued until 2000, by the means the disease was not observed in Tehran for 13 years. However, due to lack of attention and seriousness in the implementation of the project, a cat bitten an individual in Tehran was determined to be rabid. Based on data of the National Center for References and Research on Rabies, until 2003, four other rabid cats and a rabid cow, bitten by a rabid dog in the Northeastern Tehran, were found (data not Shown). According to statistics, animal biting is increasing and the most rabid animals are cows, dogs, sheep, wolves, monocots, equines, foxes, jackals, cats (Figure 6).

As stated above, controlling rabies in stray dogs (1981) in Tehran or the vaccination project for dogs in Khorasan Province in 1994-95 caused a significant reduction in the percentage of rabies in these two provinces, but the lack of continuity of the project again increased the number of rabies-infected individuals[31]. Vaccination of owned and stray dogs plays a key role in controlling rabies. There is a fluctuation in controlling rabies in animals in our country[12,31] (Figure 7).

Considering the significant activities by the healthcare networks, University of Medical Sciences and CDC for equipping and launching the rabies-prevention centers, the elimination of rabies was taken seriously since 2015. Members of the National Committee of

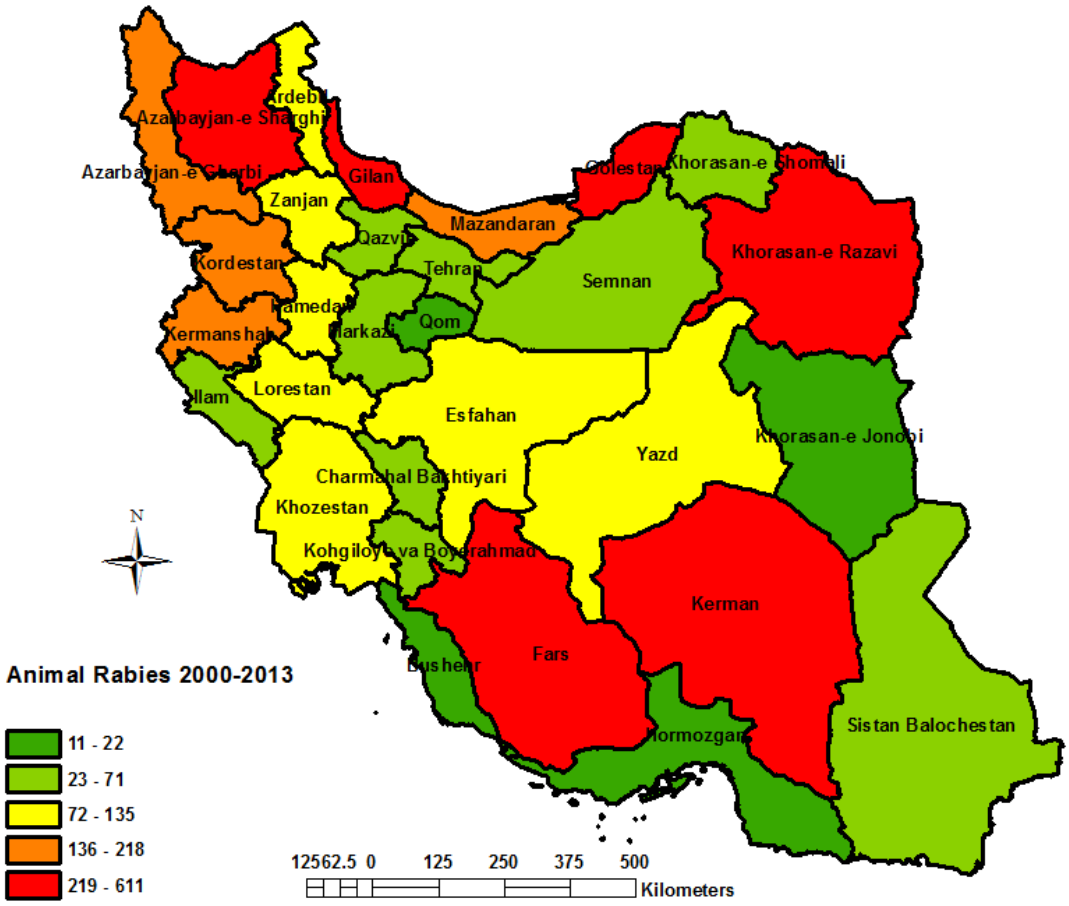

Figure 7. The distribution of rabid animals in the provinces of Iran, 2000-2013 (n=4,663)[30].

Rabies consist of the Environment Organization delegation, the Veterinary Organization, the Ministry of the Interior, and professors from universities of medical sciences, the Faculty of Veterinary Medicine of the Universities and the Pasteur Institute of Iran. They decided that each of the relevant organs should take the rabies-elimination projects seriously in their own facilities, which included the following: Elimination of human rabies by CDC, elimination of rabies in domestic animals by National Veterinary, elimination of rabies in stray animals by Organization of Rural and Urban Municipalities, elimination of rabies in the wildlife by Environmental Protection Organization.

9. Treatment and Prevention of Rabies in Iran

The animal bite has particular importance due to the risk of rabies in humans. In this regard, the community needs to be properly educated to ensure that it can take care of itself to prevent rabies and the consequences of the animal bites. In addition, by proper education of the dog keepers, proper measures should be taken in terms of vaccination of the dogs.

The principles of the animal biting control and rabies in humans should be implemented by the coordination of other organs. In many countries, rabies has been completely controlled by controlling the disease in wildlife. Recently, international organizations have been focusing on vaccination of dogs to eliminate rabies in humans. In Iran, considering the tasks of each organ and the authorities, effective measures are in place to control the disease. The main aim of the national program is to eliminate dog-induced rabies.

Individuals should be trained in such a way to refer to health centers for immediate and necessary post-exposure prevention interventions. The National Center for Reference and Research on Rabies, Pasteur Institute of Iran holds multiple seminars with the help of the CDC to train the healthcare personnel on rabies prevention and anti-rabies treatment, annually.

Disease prevention is performed by preventing the virus from entering the central nervous system that could be fatal, and it should be done under the supervision of a physician. The prevention process as follows:

1. Training is an important point about an animal-bitten individual and especially family members.
2. Local washing of the wound immediately after exposure for 15-20 minutes;
3. Effective rabies vaccination based on WHO criteria;
4. Using anti-rabies-specific immunoglobulin when needed.

Effective treatment immediately after exposure can prevent the emergence of clinical symptoms and death. Post-exposure prevention intervention is performed in 10-12 million children throughout the globe annually[35].

10. Production of Rabies Vaccine in the Pasteur Institute of Iran

Diversity of susceptible host populations in wild and domestic animals cause the big challenge to eliminate the disease. Despite recent advances in technology, medical science and paramedics, and extensive studies of the last decade, unfortunately, there is still no treatment for the bitten individuals after the onset of clinical symptoms[36].

Processes of manufacturing human and animal rabies vaccines are similar, and the WHO has published standard methodologies for producing rabies vaccine. New generation vaccines are produced under *in vitro* conditions. In 1958, the rabies virus was adapted to baby hamster kidney cells (BHK) for the first time, and this led to significant advances in cell culturing techniques[37]. Growth of the virus in cell culture facilitates a large-scale production of killed or weakened human and animal rabies vaccine. The advancement of vaccine manufacturing technologies in the past decade has led to an increase in the production of adjuvanted-vaccines for animal immunization. Cell culture methods allow to the production of standard, safe, effective, economical, and large-scale vaccines[38].

All human rabies vaccines are a type of killed or inactivated viruses, but for animals, both killed and weakened or recombinant vaccines are produced. In addition, the rabies vaccines differ in terms of the virus strain and the cell line in which they are

proliferated[39]. The cell culture process is a suitable technology for large-scale production at reasonable prices. Various stationary systems (such as culture flasks) or rotary bottles and bioreactors are cultivation methods for propagation of suspension and adherent cells. Production of the vaccine on cells requires using of a microcarrier. This technology is costly for production of animal vaccines. Therefore, suspension cells such as BHK-21 are used for the animal rabies vaccines[40].

The recombinant vaccines were designed for immunization against several infectious agents, immunization against several species or strains of a disease or to meet both goals. One of the benefits of these vaccines is to reduce the frequency of vaccination in the immunization process against various diseases. In addition, these vaccines will save time, reduce the number of visits, maintenance costs, distribution, and injection of the vaccine, as well as make the recipients more convenient and improve the immunization coverage[41]. Of course, these types of vaccines also have their own disadvantages, including immunologic interactions and undesirable complications such as allergic reactions. Moreover, due to the possibility of biologic or chemical incompatibility between the substances and antigens in the recombinant vaccines, their vaccines cannot be easily produced and extensive studies and experiments are required[41].

Since rabies is a disease that causes the highest percentage of fatalities, its bio immunity has been one of the biggest challenges in vaccine production. Therefore, researchers and manufacturers of vaccines are aiming at production of the higher-purity, more efficient and cost-effective rabies vaccines[42]. Rabies virus proteins have been expressed and produced in a variety of microorganisms, including bacteria, yeast, viruses (baculovirus, herpes virus, paramyxovirus, and herbal viruses)[43]. In most cases, the rabies virus glycoprotein has been reported as the immunogenic factor in the way that it has been able to protect the immunized animal against the deadly dose of the virus during the study. However, only cases based on poxvirus or adenovirus have been able to take the complementary steps to mass production and animal clinical trials[44] .

The inactivated rabies vaccine is relatively immunogenic and commonly used in the PEP and PrEP measurements. The quality of the immune response after vaccination greatly depends on the viral antigens used[45]. Only vaccines that are pure, immunogenic, harmless, inexpensive and able to provide long-term immunity are used for the massive immunization of animals. Therefore, development of cost-effective vaccines requires choosing the right production processes as well as adding the adjuvant to the formulation, such that it does not increase the price of the vaccine[46]. Items that are different based on the type of the vaccine are cell line used, virus strain used, cell culture system, post-production processes, formulation methods, and the immunogenicity testing of the vaccine[46].

All rabies vaccines for humans contain a rabies-inactivated virus and include the following types:

1. Human diploid cell vaccine (HDCV): In order to obtain a rabies virus suspension, lack of foreign proteins and rabies vaccine adsorbed (RVA) the proteins of the central nervous system, the virus is cultured on the WI-38 human fibroblasts cell line. The virus product is concentrated by ultrafiltration and is then inactivated in the vicinity of beta-propiolactone[47].
2. Rabies Vaccine Adsorbed (RVA)
3. Purified Chick Embryo Cell (PCEC)
4. Nerve tissues vaccine: In a few cases, this vaccine causes sensitization of the nerve tissue and even encephalitis after vaccination (0.05%).
5. Duck embryo vaccine: Duck embryo vaccine has been produced to minimize the post-vaccination encephalitis complication. In the production of this type of vaccine, the rabies virus grows in the duck's egg. Anaphylaxis reactions are not common, but the antigenic potency of the vaccine is low[48].

In Iran, vaccine used for livestock has been developed and used for past decades on neural tissue in the Pasteur Institute of Iran. The brain tissue vaccine gradually replaced the vaccine prepared on animal cell culture. The rabies vaccine has been manufactured in Iran since 2011 with machines and industrial scale at the Pasteur Institute of Iran. However, human rabies vaccine never has not developed in Iran. The vaccine has been developed in the Human Rabies Vaccine section and the initial series has been developed with standards and qualities consistent with world-class Pharmacopeia. The vaccine has been evaluated in

terms of immunogenicity by measuring the survival rate and mortality in laboratory animals immunized with the vaccine.

11. Current Status

In addition to the increase in population, public awareness, as well as the number of health centers providing rabies prevention and treatment services, and increased access to these services by the individuals in Iran, the number of animal biting cases has also increased[49]. The highest prevalence of animal bites is reported in the northern provinces, for example, Golestan and Ardebil due to the high number of owned dogs[31]. Although there has been an increasing trend in animal bites during the past 28 years (1987-2015), such that the bitten cases have increased from 35 in 1987 to 198 per 100000 of the population in 2015, the rabies cases have been steadily decreasing[31].

Obtaining comprehensive and reliable information on animal bites is difficult because most biting cases are small that the individual feels no necessity to refer to medical centers. Therefore, such cases are never reported to medical centers.

In 1994, CDC performed an animal research study on animal bites in the US. The results showed that the animal biting rate was 18 per 1000 individuals per year. It is estimated that in the US, about 2% of the total population are bitten by dogs annually (five million people), resulting in 914 daily visits to health centers[50].

In Iranian wildlife, rabies is endemic and it is important in the natural transmission of rabies to dogs and domesticated animals[31]. In Iran, in addition to dogs and wild carnivores, animals such as ferrets can be the cause of transmission of rabies to humans. Therefore, bites of these animals should be considered as similar to other wild animal cases, and post–exposure therapies, i.e. anti-rabies serum and vaccination, should be performed[31].

According to the Zoonosis Control Department of the Ministry of Health, the ferret has been responsible for 3% rabies cases in humans in southern Iran over the last 10 years. In Iran, 62% of animal bites have been reported in rural areas and 38% in urban areas[31].

Although it has been a century since Louis Pasteur discovered the rabies vaccine, the disease is still endemic in most countries of the world from polar to tropical regions because of the diversity of susceptible host populations in wild and domestic animals such as carnivores, herbivores, rodents, and bats. Despite recent advances in technology, medical science and paramedics, and extensive studies of the last decade, unfortunately, there is still no treatment for the bitten individuals after the onset of clinical symptoms, such that every 10 minutes one fatality is reported due to rabies (more than 90% of which are in developing countries in Africa and India)[51,52].

Health care shortages, high prices, and the complexity of the production of anti-rabies vaccines often inhibit the prevention of human rabies in developing countries. Cell culture vaccines can be effective when the wound is properly cared for and immunoglobulin is used, but high costs, need for follow up doses and cryogenic cycles are the production problems of these vaccines, especially in developing countries[53].

Inactivated vaccines are currently the most commonly used vaccines in the world. Cell-based vaccines are used in developed countries where rabies is a serious problem. Sometimes, chemical inactivation deteriorates the structure of viral antigens and increases the risk of immunogenicity[53].

Due to the attempts made by the relevant organs in Iran, and by assigning the rabies prevention treatment facilities and raising the awareness and performance of the personnel of these centers as well as public training, the timely reference of the animal-bitten subjects to the prevention centers and completion of the vaccination course is now common. We hope that someday, by the proper coverage of immunization of the wildlife, owned and stray dogs, we can observe selective immunization in animal-bitten human subjects.

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Conflict of Interest

The authors further declare that, they have no conflict of interest.

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