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THE HISTORY OF RABIES IN TUNISIA AND THE DEVELOPMENT OF THE RABIES VACCINE

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REVIEW ARTICLE

ABSTRACT

Over 55,000 people are killed by rabies each year, which continues to be a hazard to worldwide public health. Due to the number of years lost as a result of the disease, rabies is listed as the sixth most significant infectious disease. An illustrious history of more than 120 years has gone into the improvement of rabies vaccination for animals and humans. This review describes the different types of rabies virus vaccines and the development of replication-defective or monocyclic live rabies virus-based vectors for use as single-dose human rabies vaccinations using reverse genetics methods.

Key words: Rabies virus, Vaccination, Vaccines, genetics methods.

INTRODUCTION

Rabies is a fatal infection of the nervous system. It is due to the multiplication in the nerve centers of a neurotropic rhabdovirus “the rabies virus”. It causes encephalomyelitis with a fatal outcome and is usually accompanied by aggression, excitement, or paralysis. It is transmissible to humans following the breach of the omucous skin barrier by biting, scratching, or licking a rabid animal, most often a dog (Dacheux.L & Bourhy.H, 2011). Indeed, more than 3.3 billion people live in rabies enzootic areas, and every year, more than 55,000 people die of rabies. 99% of them are inhabitants of the Third World. Specifically, an estimated 95% of these deaths occur in Africa and Asia (World Health Organization,2004) (Yousaf, MZ et al,2012).

There are many epidemiological cycles in nature, each associating a type of Lyssa virus with a different species. Canine rabies, which typically affects dogs but rarely cats or other domestic animals. When the rabid dog strays far from its point of origin, it can contaminate animals or humans. Canine rabies is widespread across the globe: in Africa, South America, Asia, and in a few rare European countries, such as Turkey, where it is enzootic (Fekadu, M, 1993). Rabies in wild animals can infect many wild species, which will ensure its transmission, even if they are most often carnivores. The species playing a predominant role varies by country. We can mention the red fox (Vulpes vulpes) for western and central Europe, the polar fox (Alopex lagopus) for Greenland, and the wolf for some

regions of Iran (Blancou, J et al,1997). It has been shown that Chiropteran rabies exists in Latin America and the United States, but it has also been reported in Europe, particularly in Spain and Germany. It is also found in India and Thailand (Bruyère-Masson et al, 2001). This review describes the different types of rabies vaccines and the development of the vaccines during these years.

History of rabies in Tunisia

Rabies was described for the first time in Tunisia by Ibn Jazzar in the tenth century. There is no written trace of this disease between the 10th and 18th centuries. The first cases that appeared around 1870 in Tunisian capital were immediately linked to the arrival of European migrants. Its importation into Tunisia is quite likely by two routes of entry: land from Algeria or sea from the European coastal cities Malta or Sicily, for example (Néfissa, K. B et al, 2007). The evolution of rabies virus in Tunisia from 2017 to 2019 is summarized in the following figure:

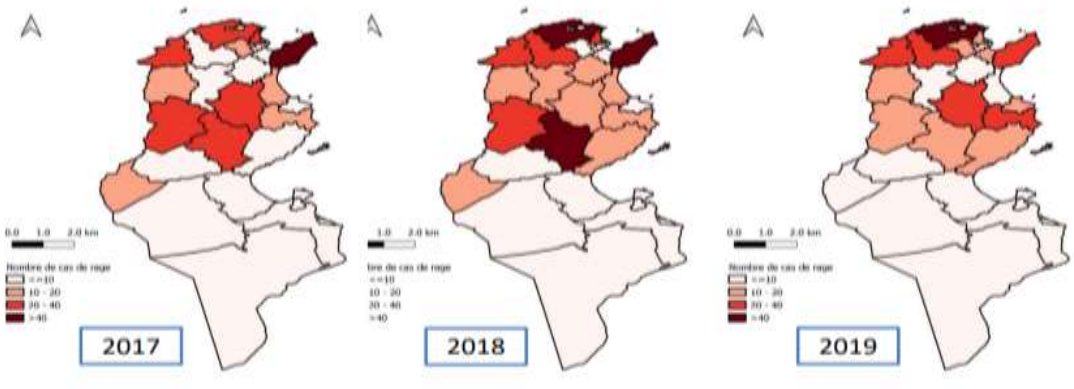

Figure 1: Epidemiological situation of animal rabies in Tunisia: annual evolution from 2017 to 2019. (Emna, B. 2021).

Rabies virus structure

Rabies virus belongs to the Rhabdoviridae family and the Lyssavirus genus. Rabies viruses have a form of shell that is approximately 100-300 nm in length by 75 nm in diameter (Figure 2).

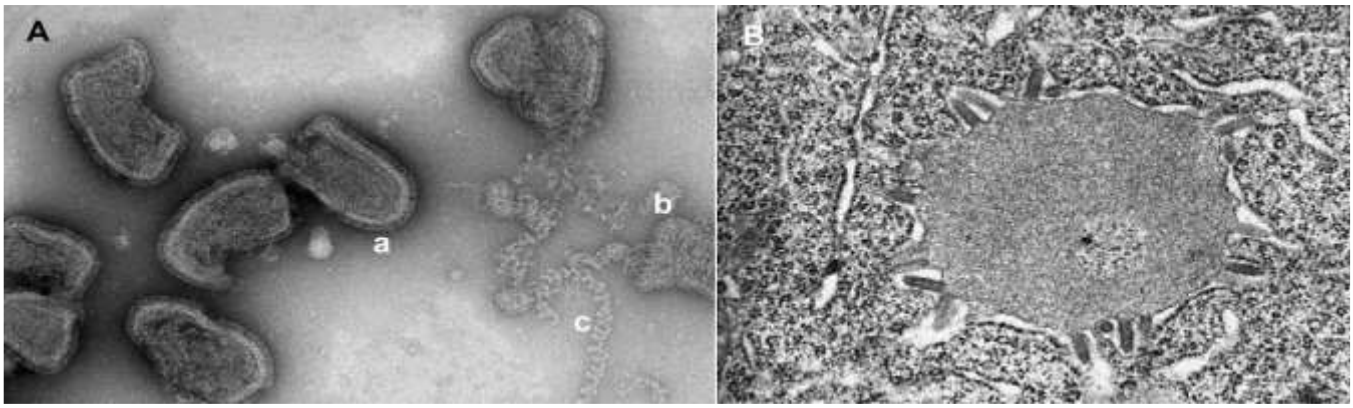

Figure 2: Electron microscopy visualizations of the rabies virus.

A) Microscopy image electron of rabies viral particles (Pasteur strain). a: Virus particle. b: Viral

nucleocapsid unfolding, c: Free nucleocapsid.

B) Neuron of a dog inoculated with the rabies virus. Cutting Ultra-thin cell body shows viral matrices M and budding virion, magnification original x 62500 (Schaechter, M., et al.1999) .

The rabies virus comprises a phospholipid membrane containing a glycoprotein G in the external position, which associates in the form of trimers to form spicules, and a matrix protein M in its internal position. Inside the virus is the nucleocapsid, which is formed by the association between viral RNA of negative polarity and three viral proteins: nucleoprotein (N), viral RNA polymerase (L), and phosphoprotein (P) (Figure 3).

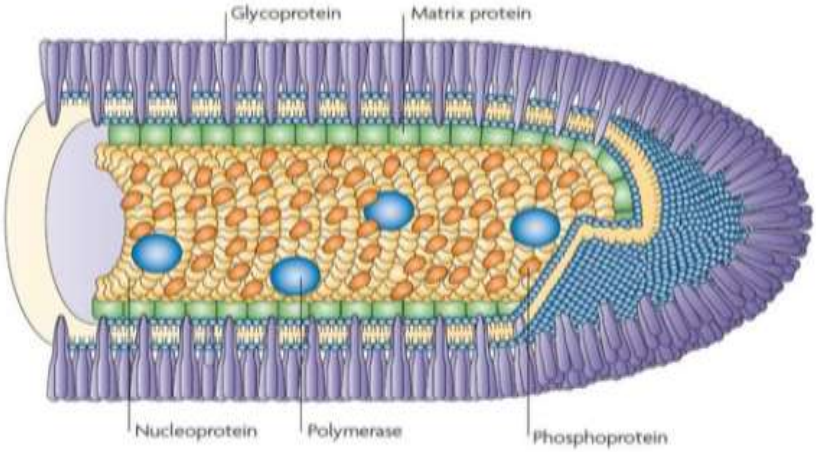

Figure 3: Schematic representation of the rabies virus.

The nucleocapsid is made up of following proteins: nucleoprotein, phosphoprotein and polymerase. The internal face of the envelope is lined by the M protein, while the outer face is covered by the glycoprotein with which it is associated as a trimer in the form of a spicule (Schnell. MJ, et al, 2010).

The different types of rabies vaccines

Rabies vaccines since Louis Pasteur have undergone several advances: initially based on the principle of attenuation, they are favored by cell culture and revolutionized in recent years by the contributions of molecular biology and genetic engineering.

Vaccines produced on nerve tissue: These vaccines are the result of the first vaccination trials against rabies. The first vaccine developed by Louis Pasteur and his collaborators was based on shredded rabbit marrow infected with the rabies virus. Vaccination was done by the administration of multiple doses and increasing virulence in the abdominal wall. Despite its effectiveness, this vaccine has several side effects such as immuno-allergic reactions such as encephalomyelitis or polyneuritis, as well as a high risk of pathogenicity due to the virulence of the last doses, which are the most virulent (Rotivel.Y, et al, 2002). Despite all the improvements made to vaccines prepared on animal brains, the problems of safety and low immunogenic power still remain limiting, which has prompted the WHO to recommend since 1992 that these vaccines should no longer be used. This was not until

now respected by various developing countries because of the low cost of these vaccines, which is within the reach of their poor populations.

Vaccines produced in embryo culture: Vaccines produced on avian embryo cultures of early explants represent the second generation of rabies vaccines. Examples include vaccines produced on purified and concentrated duck embryos as well as the vaccine produced with the viral strain Flury HEP on chicken embryonic tissue (Rotivel.Y, et al, 2002).

Vaccines produced in cell culture: Vaccines produced on cell culture have been considered since their appearance in 1960 the most effective and safe vaccines against rabies. Since then, they have been the most recommended by the WHO because of their purity, their high immunogenicity, and their administration protocol consisting of a reduced number of injections compared to other types of vaccines (World Health Organization, 2002). These vaccines include:

- Vaccines prepared on human diploid cells (HDCV): developed in 1972 by Bahmanyar and H. Koprowski and his team using the Pitman-Moore L503 strain or the Flury strain. This type of vaccine has been administered to more than 1.5 million people worldwide (Rotivel, Y., et al, 2002).
- Vaccines prepared on chicken embryo fibroblasts (PCEC): prepared from the attenuated strain Flury HEP.
- Vaccines produced on monkey kidney cells (VERO cell): this vaccine uses the Wistar strain of the rabies virus in culture on monkey kidney cells of the VERO line (Rotivel.Y, et al, 2002).

It has been noted that rabies vaccines produced in cell culture are sometimes associated with allergic reactions to HDCV vaccines following the formation of IgE directed against human serum albumin modified by β-propiolactone used for inactivation of the virus. These reactions are transient and safe. The only real limit for the use of these vaccines is currently their very high cost for developing countries. In this context, Thai teams have developed multi-site intradermal vaccination protocols, the principle of which is to use less than half the dose of antigens. This approach, validated by the WHO, guarantees the production of protective immunity faster and at a lower cost than that of conventional cell culture vaccines.

Vaccines based on reconstructed live attenuated viruses: The contribution of molecular biology and genetic engineering in the development of rabies vaccination is undeniable. They make it possible to obtain new vaccines attenuated by directed mutations and offer the possibility of obtaining vaccines that replace those based on inactivation in post- and pre-exposure applications. The latter exploit the immunogenic properties of live vaccines to elicit an effective innate and adaptive response. In fact, reverse genetics and the ability to manipulate the rabies virus genome make it possible to obtain new viral formulas that are very promising as new generations of rabies vaccines. In addition, new technologies to construct recombinant viral vectors capable of expressing immunogenic viral particles, as well as DNA vaccines, would constitute a new wave of rabies vaccines.

Vaccines based on recombinant attenuated viruses: The relatively simple molecular architecture of the viral genome facilitates its manipulation to attenuate the rabies virus while maintaining its ability to induce inflammation and subsequently elicit rabies immune responses. In this context, viruses that are deleterious or have added genes encoding the rabies proteins M, P, and G or other genes have shown great efficacy in vaccination against rabies.

Rabies vaccines based on rabies virus with the phosphoprotein P gene deleted: This approach was based on the properties of phosphoprotein P rabies as an essential non-enzymatic element in the polymerase complex through the interaction between viral proteins and those involved in virus replication, as well as as an antagonist of INF α /β and inhibitor of its signaling pathway in infected cells (Brzozka, K., Finke, S., & Conzelmann, K. K, 2005) (Brzozka, K., Finke, S., & Conzelmann, K.K, 2006). It has been shown that a highly attenuated rabies strain deleted from the P gene is unable neither to replicate at the level of the infected cell nor to spread to the central nervous system (Morimoto, K., et al, 2005) (Shoji, Y., et al, 2004). These pathogenic viruses have retained their immunogenicity: they are in fact capable of inducing a strong Th1 response and producing IgG2a antibodies (Cenna, J.,et al, 2008). This response induced in mice immunized by this construction was shown to be ten times more protective than vaccination with a virus inactivated by UV. These viruses can be used for pre-exposure vaccination but not as post-exposure treatment because of the slow induction of the protective response.

Rabies vaccines based on rabies virus with the matrix protein M gene deleted: Rabies viruses with the matrix protein M gene deleted retain their replicative capacities in infected cells and therefore their ability to express themselves for a long period of time while remaining unable to reach the central nervous system (Cenna, J., et al, 2009) (Ito, N., et al 2004). These modified viruses also show the ability to induce a humoral response through the production of IgG2a which is able to eliminate free virus or cell-associated viruses by different effector mechanisms (Burton, D. R, 2002). These antibodies, detectable 5 days after inoculation with low doses, are characterized by high avidity compared to those induced by a vaccine prepared on human diploid cells and with a short induction time.

Vaccines based on replication-defective viruses expressing two copies of glycoprotein G: Vaccination based on replication-defective viruses expressing two copies of glycoprotein G presents an advantageous approach combining the expression at high levels of the major antigenic determinants of the rabies virus: glycoprotein G, while maintaining its safety (Cenna, J., et al, 2009). These viruses are also immunogenic, and the IgG2a antibodies they induce are 100% protective following a challenge by the pathogenic rabies virus (Cenna, J., et al, 2008).

Rabies virus vaccine with foreign genes: Based on the fact that the recruitment and activation of dendritic cells promotes the protective immune response against the rabies virus, a group of researchers proceeds by cloning in the rabies virus recruitment genes or cell activating molecules genes such as GM. (granulocyte macrophage colony stimulating factor), (macrophage derived cytokine) genes, etc. Intramuscular infection of mice with these modified viruses recruits or activates more dendritic cells secreting INF alpha as well as cells B peripheral, which results in high levels of anti-rabies neutralizing antibodies that significantly protect mice challenged intracerebrally by the virulent virus (Wen, Y., et al, 2011).

Subunit protein vaccines: As part of the research to develop rabies vaccines, several groups have tested vaccines administered orally. These vaccines are based on rabies glycoprotein G purified from the virus and expressed in different expression systems.

Protein vaccine obtained by the immunosome technique: The idea of this vaccine is based on the binding of the rabies G protein to a phospholipid vesicle called liposome, which will promote the immunogenicity of this protein. Indeed, this technique allows correct exposure of the immunodominant epitopes involved in the induction of anti-rabies neutralizing antibodies and also induces a specific cellular response (Sureau, P., & Perrin, P, 1989).

Vaccine based on G protein expressed in an insect cell infected with recombinant baculovirus vectors: The rabies G protein gene expressed by a baculovirus-vector in an insect cell had proven itself as a possible vaccine approach against rabies. Indeed, a baculovirus transfer vector, pAcYM1, derived from the nuclear polyohydrosis virus of Autographa California (AcNPV), harboring the rabies glycoprotein gene, was used to infect the Spodoptera frugipedra cell line. The expressed protein exhibited conserved antigenic and immunogenic properties compared to the native protein (Prehaud, C., et al,1989). The limits of this approach are, on the one hand, the high cost of protein purification and, on the other hand, the small size of the proteins produced, hence their high susceptibility to post-translational modifications, risking losing the functional structure essential for their antigenicity (Ertl, H.C.J,2009).

Vaccine based on rabies G proteins expressed in yeast cultures: Rabies virus G glycoproteins can be produced by yeast cells transfected with cDNA encoding the G protein. Proteins expressed by this system are sometimes misfolded and therefore weakly immunogenic (Sakamoto, S., et al,1999).

Protein vaccine expressed on mammalian cells: Mammalian cells such as MDBK (Madin Darby Bovine Kidney) transformed with plasmid constructs encoding the soluble forms of rabies glycoprotein G express the recombinant protein, which has been shown to be immunogenic following injection (Gupta, P. K., et al, 2005).

Protein vaccine expressed by transgenic plants: Based on the idea of the edible vaccine, several research groups have carried out the transgenesis of several plants by bacterial vectors carrying the gene construct coding for the rabic glycoprotein G (McGarvey, P.B., et al, 1995). These constructs may encode the entire G protein or major antigenic determinants of the glycoprotein recognized by neutralizing antibodies (Yusibov, V., et al, 2002). The immunogenicity of these protein entities expressed and isolated from plants has been successfully tested - in humans who have consumed these transgenic vaccine plants. Nevertheless, despite the ease of obtaining large quantities, these vaccines pose a problem of tolerance and readiness in the digestive system and also of the immune reaction in the intestines to the vaccine antigen.

DNA vaccines: DNA vaccines consist of plasma vectors responsible for expressing the vaccinating antigen. These vaccines have been shown to be very stable and it induces a response against the desired antigen. These vaccines are also easy to generate, and their production is simpler and more cost effective than that of purified proteins or mammalian tissue cell culture vaccines. The efficacy of DNA vaccines is confirmed in experimental animals as well as in dogs, cats, and primates (Bodles-Brakhop, A. M., et al, 2009) (Lodmell, D. L., et al.2006). It strongly depends on the route of administration: Indeed, the response to vaccination by the intradermal route is more effective than that by the intramuscular route (Tesoro-Cruz, E., et al. 2008). However, DNA vaccinations have several limitations, such as the slow induction of an immune response and the induction of mild local reactions.

RNA-based vaccines: Recently, an Indian group has developed an RNA-based rabies vaccine: Indeed, self-replicating RNA coding for rabies glycoprotein G was developed using the RNA replicon of the Sundbis virus. Immunization of mice with RNA transcribed in vitro (Sin-Rab-GNRA) elicited two responses: cellular and humoral IgG type similar to those induced by a DNA vaccine and protective responses following the challenge by the CVS strain (Saxena, S., et al, 2009).

CONCLUSION

Countless lives have been saved since human evolution from neuroscience rabies vaccine was introduced about 120 years ago. The complex process of preventing human from rabies virus including controlling rabies in wildlife as well as feral and domesticated dogs and the development of new vaccines; The challenge for the next five years is to produce a new, affordable, and effective rabies vaccine**.**

REFERENCE

[1] Dacheux, L., & Bourhy H. **(2011)**. Le diagnostic de la rage. Revue Francophone des Laboratoires, 430, 33-40.

[2] World Health Organization. **(2004)**. WHO Expert Consultation on Rabies. 1st report. WHO Technical Report Series, No. 931. Geneva, Switzerland: World Health Organization;

[3] Yousaf, M. Z., Qasim, M., Zia, S., Khan, M. U. R., Ashfaq, U. A & Khan, S. **(2012)**. Rabies molecular virology, diagnosis, prevention and treatment. *Virology Journal*, 9(50), 1-5.

[4] Fekadu, M. **(1993)**. Canine rabies. *The Onderstepoort journal of veterinary research*, 60(4), 421.

[5] Blancou, J., Aubert, M. F. A., Blancher, G., Nordmann, R., Rerat, A., & Laroche, C. **(1997)**. Transmission du virus de la rage: importance de la barrière d'espèce. *Discussion. Bulletin de l'Académie nationale de médecine*, 181(2), 301-312.

[6] Bruyère-Masson, V., Arthur, L., Barrat, J., & Cliquet, F. **(2001)**. Les données actuellement disponibles sur les populations de Chiroptères autochtones, leur situation épidémiologique au regard de la rage. *Bulletin Epidémiologique Hebdomadaire*, (39).

[7] Néfissa, K. B., Moulin, A. M., & Dellagi, K. **(2007)**. La rage en Tunisie au XIXC siècle: recrudescence ou émergence? *Gesnerus*, 64, 173-192.

[8] Emna, B. **(2021)**. Epidemiological situation Rabies in Tunisia. https://rr-africa.woah.org/wp content/uploads/2021/03/situation-epidemiologique-de-la-rage-en-tunisie.pdf.

[9] Schaechter, M., Medoff, G., & Eisenstein, B. I. **(1999)**. Microbiologie et pathologie infectieuse. *De Boeck édition.*

[10] Rotivel, Y., Goudal, M., Perrin, P., & Tordo, N. **(2002)**. Une histoire de la vaccination contre la rage. *Virologie*, 6, 89-104.

[11] World Health Organization. **(2002)**. Core information for the development of immunization policy: 2002 update. Geneva: WHO. Retrieved July, 5, 2012.

[12] Brzozka, K., Finke, S., & Conzelmann, K. K. (**2005**). Identification of the rabies virus alpha/beta interferon antagonist: phosphoprotein P interferes with phosphorylation of interferon regulatory factor 3. *Journal of Virology*, 79,7673-7681.

[13] Brzozka, K., Finke, S., & Conzelmann, K.K. (**2006**). Inhibition of interferon signaling by rabies virus phosphoprotein P: activation-dependent binding of STAT1 and STAT2. *Journal of Virology*, 80, 2675-2683.

[14] Morimoto, K., Shoji, Y., & Inoue, S. **(2005)**. Characterization of P gene-deficient rabies virus:

propagation, pathogenicity and antigenicity. *Virus Research*, 111, 61-67.

[15] Shoji, Y., Inoue, S., Nakamichi, K., Kurane, I., Sakai, T., & Morimoto, K. **(2004)**. Generation and characterization of P gene-deficient rabies virus. *Virology*, 318, 295-305.

[16] Cenna, J., Tan, G.S., Papaneri, A.B., Dietzschold, B., Schnell, M.J., & McGettigan, J.P. **(2008)**. Immune modulating effect by a phosphoprotein-deleted rabies virus vaccine vector expressing two copies of the rabies virus glycoprotein gene. *Vaccine*, 50, 6405-6414.

[17] Cenna, J., Hunter, M., Tan, G.S., Papaneri, A.B., Ribka, E.P.,Schnell, M.J., Marx, P.A., & McGettigan, J.P. **(2009)**. Replication-deficient rabies virus–based vaccines are Safe and immunogenic in mice and nonhuman primates. *The Journal of Infection Diseases*, 200, 1251-1260.

[18] Ito, N., Sugiyama, M., Yamada, K., Shimizu, K., Takayama-Ito, M. U. T. S. U. Y. O., Hosokawa, J., & Minamoto, N. **(2004)**. Characterization of M gene-deficient rabies virus with advantages of effective immunization and safety as a vaccine strain. *Microbiology and immunology*, 49(11), 971-979.

[19] Burton, D. R. **(2002)**. Antibodies, viruses and vaccines. *Nature Reviews Immunology*, 2(9), 706-713.

[20] Wen, Y., Wang, H., Wu, H., Yang, F., Tripp, R.P., Hogan, R.J., & Fu, Z.F. **(2011)**. Rabies virus expressing dendritic cell-activating molecules enhances the innate and adaptive immune response to vaccination. *Journal of Virology*, 85, 1634–1644.

[21] Sureau, P., & Perrin, P. **(1989)**. The use of immunosome technology for vaccines against rabies and other viral diseases. *European Journal of Epidemiology*, 5(3), 275-278.

[22] Prehaud, C., Takehara, K., Flamand, A., & Bishop, D.H.L. **(1989)**. Immunogenic and protectiveproperties of rabiesvirusglycoproteinexpressed by baculovirusvectors. *Virology*, 173, 390-399.

[23] Ertl, H.C.J. **(2009)**. Novel vaccines to human rabies. PLoS Neglected Tropical Diseases, 3(9), e515.

[24] Sakamoto, S., Ide, T., Tokiyoshi, S., Nakao, J., Hamada, F., Yamamoto, M., Grosby, J.A, Ni, Y., & Kawai, A. **(1999)**. Studies on the structures and antigenicproperties of rabies virus glycoprotein analogues produced in yeastcells. *Vaccine*, 17, 205-218.

[25] Gupta, P. K., Sharma, S., Walunj, S. S., Chaturvedi, V. K., Raut, A. A., Patial, S., Rai, A., Pandey, K. D., & Saini, M. **(2005)**. Immunogenic and antigenic properties of recombinant soluble glycoprotein of rabies virus. *Veterinary microbiology*, 108(3), 207-214.

[26] McGarvey, P.B., Hammond, J., Dienelt, M.M., Hooper, D.C., Fu, Z.F., Dietzschold, B., Koprowski, H., & Michaels, F.H. **(1995)**. Expression of the rabies virus glycoprotein in transgenic tomatoes. Biotechnology *(N Y)*, 13, 1484-1487.

[27] Yusibov, V., Hooper, D.C., Spitsin, S. V., Fleysh, N., Kean, R. B., Mikheeva, T., Deka, D., Karasev, A., Cox, S., Randall, J., & Koprowski, H. **(2002)**. Expression in plants and immunogenicity of plant virus-based experimental rabies vaccine. *Vaccine*, 20(25), 3155-3164.

[28] Bodles-Brakhop, A. M., Heller, R., & Draghia-Akli, R. **(2009)**. Electroporation for the delivery

of DNA-based vaccines and immunotherapeutics: current clinical developments. *Molecular Therapy*, 17, 585-592.

[29] Lodmell, D. L., Ewalt, L. C., Parnell, M. J., Rupprecht, C. E., & Hanlon, C. A. **(2006)**. One-time intradermal DNA vaccination in ear pinnae one year prior to infection protects dogs against rabies virus. *Vaccine*, 24(4), 412-416.

[30] Tesoro-Cruz, E., Calderón-Rodríguez, R., Hernández-González, R., Blanco-Favéla, F., & Aguilar-Setién, A. **(2008)**. Intradermal DNA vaccination in ear pinnae is an efficient route to protect cats against rabies virus. *Veterinary research*, 39(2), 1-11.

[31] Saxena, S., Sonwane, A. A., Dahiya, S. S., Patel, C. L., Saini, M., Rai, A., & Gupta, P. K. **(2009)**. Induction of immune responses and protection in mice against rabies using a self-replicating RNA vaccine encoding rabies virus glycoprotein. *Veterinary microbiology*, 136(1), 36-44.