Rabies Vaccines: Its Role, Challenges, Considerations and Implications for the Global Control and Possible Eradication of Rabies


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Abstract: This review reports on the rabies vaccines: Its role, considerations and implications for the global control and possible eradication of rabies. Attempts to control human rabies have a long history; animal and human vaccines provide efficient weapons for prevention. Vaccines are one of the most effective public health interventions. Vaccines are the basis of the medical and veterinary medical future. Rabies vaccine is made from killed rabies virus. Rabies vaccine can prevent rabies. It is offered to people at high risk of exposure. The primary intention of vaccine is to produce stimulation to the cellular immune system, via the production of antibodies. Methods for Rabies Virus (RABV) manipulation have changed fundamentally from random attenuation to defined modifications. In 2001, WHO issued a resolution for the complete replacement of nerve tissue vaccines by 2006 with cell-culture rabies vaccines? In recent years, purified and concentrated Vero cell rabies vaccines using the 3aG and CTN-1 strains have been developed. The Purified Vero Rabies Vaccines (PVRV), is also being developed to meet the increasing demand for human rabies vaccine. However, for animals, all fixed RABV strains recommended by WHO, such as PVRV, Challenge Virus Standard (CVS), Flury-Low Egg Passage (LEP), High Egg Passage (HEP), Evelyn-Rokitnicki-Abelseth (ERA), and SAD variants, have been successfully used in industrialized countries, where rabies is well controlled. Any potent rabies vaccine will protect against rabies. A vaccine, like any medicine, is capable of causing serious problems, such as severe allergic reactions, though the risk of causing serious harm, or death, is extremely small and very rare. As international concerns increased, several corrective actions have been implemented in many countries since 2005, which aimed at improving vaccination protocols and a consistent vaccination strategy aiming to eliminate the residual focus. However, we should bear in mind that vaccination is still the key to prevent rabies in small animals and transmission to human beings. It is hoped that the various strategies, well coordinated and corrective actions and initiatives for global control of rabies, to make important contributions in stemming the magnitudes, roles and implications of vaccines for global control and possible eradication of rabies and other rabies-related viruses which poses threat to global public health.

Keywords: Elimination, global control, possible eradication, rabies vaccines, vaccination

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INTRODUCTION

Rabies, being a major zoonotic disease, significantly impacts global public health. It is invariably fatal once clinical signs are apparent. The majority of human rabies deaths occur in developing countries (Nagarajan et al., 2008). Rabies is one of the oldest known diseases of mankind. Despite these continuing problems, there has been tremendous progress in the control of rabies. Cheap and safe vaccines for animals as well as humans have been developed over the years. The development of the first rabies vaccine by Pasteur was surely hoped to eliminate or at least drastically reduce the incidence of rabies. It has been more than 100 years since this first vaccine was developed for pre-exposure vaccination and post-exposure treatment, yet new patterns and trends of rabies infection present a major challenge and concern for animal scientists, veterinarians, epidemiologists and virologists alike. Although a vaccine-preventable disease, effective rabies prevention in humans with category III bites requires the combined administration of Rabies Immunoglobulin (RIG) and vaccine (Nagarajan et al., 2008). Rabies vaccine can prevent rabies. It is usually given to people at high risk of rabies to protect them if they are exposed. It can also prevent the disease if it is given to a person after they have been exposed. Since the first crude nerve tissue vaccine, numerous other rabies vaccines for human use have been developed and used with varying degrees of effectiveness and safety. When used appropriately, new cell culture vaccines provide nearly 100% protection with a high degree of safety.

Furthermore, vaccines are one of the most effective public health interventions (Black, 2009). Vaccines are the basis of the medical and veterinary medical future. The remarkable growth of vaccine biotechnology continues apace in basic science discovery, product development, market introduction, and adoption into immunization programs (NFID, 2009). Typically, the vaccines are injected into the body; subcutaneously (under the skin) or intramuscularly (in the muscle). Vaccination makes use of the immune system to combat virus infections. The primary intention of vaccine is to produce stimulation to the cellular immune system, via the production of antibodies. Antibodies attach onto the virus and render it inactive and harmless. It is through this stimulation and resultant production of antibodies that the body is now prepared for a possible 'attack' by 'foreign invaders' later down the line. These invaders are typically known as bacteria or viruses. This immunity will later provide protection without having to go through the disease itself. It is a bit like the vigilante minutemen always on guard for a possible attack (Blanco, 2008).

Immunity to a viral infection depends on the development of an immune response to antigens present on a virally infected cell or on the surface of the virion itself. Immune responses to internal antigens play little role in immunity (Hunt, 2000). Often it is the glycoprotein to which the population has not been exposed and thus, against which the population has no immunity. Surface glycoproteins are often referred to as protective antigens (Hunt, 2000). To make a successful vaccine, it is likely that the identity of these antigens will have to be known unless the empirical approach of yesteryear is to be followed. There may be more than one surface glycoprotein on a virus and one may be more important in the immune response than the other (Hunt, 2000). Neutralizing antibody that blocks infectivity is required of a successful vaccine. This usually results from antibody combining with surface protein that would otherwise bind to 2 surface receptor of cell. Complement can also lyse enveloped virions.

The two forms of vaccines available are the Modified-Live Vaccines (MLV) and the killed vaccines. However, for obvious reasons, the rabies vaccine is a killed product. It cannot cause rabies. MLVs are the viruses that were once alive and now have been chemically attenuated (altered) so that they are still recognized by the body but are, theoretically, not able to cause the full blown clinical disease (O'Driscoll, 2008). Some of the newest types of vaccines are called subunit and naked DNA vaccines. DNA vaccination is a novel immunization strategy that has great potential for the development of vaccines and immune therapeutics. This strategy has been highly effective in mice, while less immunogenic in nonhuman primates and humans (Muthumani et al., 2009). Without going into the intricacies of their production, they involve techniques used in genetic engineering. Subunit vaccines generally will insert a viral or bacterial DNA section into the DNA from yeast, which is allowed to reproduce in large quantities. The protein intended for inclusion in the vaccine is then separated from the yeast cells. In the case of naked DNA vaccines, the viral or DNA gene is first reproduced, then spliced into a plasmid (which is essentially free DNA, widely used in recombinant technology), reproduced in bacteria or cells, and then separated from them for inclusion in the vaccine (McRearden, 2008). Recombinant gene vaccines can also be produced via these methods for instance; vaccinia virus (VV)-rabies is now an exclusively recombinant vaccine. One of the major concerns with these methods is the unpredictability and interaction of the final vaccine product with the proteins or DNA of the host (McRearden, 2008).

In the past several years, millions of rabies-virus, vaccine-laden baits has been distributed over rural and urban areas in Western Europe, Eastern Canada, and the U.S. for wildlife rabies control and a number of attenuated and recombinant rabies vaccines have been developed. Oral Rabies Vaccines (ORV) have been successfully
developed for red, Arctic, and gray foxes; coyotes, raccoon dogs, raccoons, skunks, and domestic dogs. Today, the combination of serum and vaccine is the recommended standard for prophylaxis in human rabies exposure (Lyles and Rupprecht, 2007). In recent time, new cytokines are identified, innate and induced immune regulatory pathways unraveled, novel adjuvants and antigen constructs prove effective, and recently-licensed products achieve high coverage, already yielding noticeable decreases in disease incidence (Morrow and Weiner, 2008; NFID, 2009). New strategies like adjuvantation are explored in the development of new, prophylactic vaccines against challenging diseases and/or to target specific populations. Adjuvant Systems (ASs) are tailor-made combinations of conventional adjuvants with immunomodulators designed to enhance protective immune responses to specific vaccine antigens (Garçon et al., 2006; Garçon, 2009). One can envision a growing number of challenging maladies - including chronic, non-infectious, and neoplastic - that may become vaccine-preventable or vaccine-treatable in the years ahead (NFID, 2009).

Given the progress in biotechnology, modern generations of rabies vaccines will have both maximal efficacy and safety while still being affordable in regions with enzootic dog rabies (Lyles and Rupprecht, 2007). Attempts to control human rabies have a long history; animal and human vaccines provide efficient weapons for prevention. Oral vaccination of wildlife with recombinant rabies virus vaccines began to reduce the incidence of rabies among foxes and raccoons. Vaccination of stray dogs also led to the eradication of rabies in countries where dog rabies is the sole source of human exposure. However, concerns about vaccine safety, both real and imagined, can and have undermined what would otherwise be effective public health interventions (Black, 2009). This review therefore reports on the rabies vaccines: its role, considerations and implications for the global control and possible eradication of rabies.

RESULTS AND DISCUSSION

Historical background of vaccines and vaccinations: The first universally applied vaccine, vaccinia virus, is of obscure origin. The birth of vaccinations came when the English doctor Edward Jenner in Berkeley, Gloucestershire, discovered that the people who worked closely with cows seemed to be less susceptible to smallpox (Alan, 2005). In 1796, Jenner discovered vaccination using vaccinia virus, the agent of cowpox, since people who got cowpox were known to have protective immunity against the much more virulent smallpox (Hunt, 2000). He injected small amounts of the cowpox crusts from Blossom into healthy individuals (Vaccinated including his own son) and then challenged with virulent smallpox and found these people to also be less susceptible to smallpox. For instance, he inoculated 8-year-old James Phipps with purulent material taken from a cowpox pustule on the hand of milkmaid Sarah Nelmes and introduced it into an incision on the boy's arm. Jenner subsequently proved that the boy became immune to smallpox. He published the work in 1798, coined the word vaccine from the Latin vacca for cow, and designated the process as vaccination (Graham and Crowe, 2007).

Although Jenner is commonly given the credit for vaccination, variolation, the practice of deliberately infecting people with smallpox to protect them from the worst type of the disease, had been practiced in China at least 2000 years previously (Alan, 2005). In 1774, a farmer named Benjamin Jestly had vaccinated his wife and two sons with cowpox taken from the udder of an infected cow and had written about his experience. Jenner was the first person to deliberately vaccinate against any infectious disease (i.e., to use a preparation containing an antigenic molecule or mixture of such molecules designed to elicit an immune response) (Alan, 2005). Then, during the American Civil War, Louis Pasteur, an accomplished microbiologist, was able to change the vaccines he was using enough that some of the harmful effects were diminished. He was famous for his work in cattle where he was able to prove that vaccines could protect against the deadly disease, anthrax (O'Driscoll, 2008).

In 1885, Pasteur experimented with rabies vaccination, using the term virus (Latin for 'poison') to describe the agent. Although Pasteur did not discriminate between viruses and other infectious agents, he originated the terms virus and vaccination (in honour of Jenner) and developed the scientific basis for Jenner’s experimental approach to vaccination (Alan, 2005). Pasteur’s research on rabies is perhaps the most well known historical achievement in the field (Lyles and Rupprecht, 2007). First, through adaptation of street (wild-type) virus to laboratory animals, he was able to change its properties. Today, one could apply the term attenuated to his fixed virus strains. Second, Pasteur and his team developed concepts and experimental approaches to the first protective vaccination against rabies (Koprowski, 1985 cited in Lyles and Rupprecht, 2007). Desiccated spinal cords from rabies virus-infected rabbits became the first rabies vaccine, and they were supposedly safe, although now it is known that the fixed viruses from which these vaccines were derived were not apathogenic but could actually cause the disease. July 6, 1885, is a milestone in the history of rabies. On that day, 9-year-old Joseph Meister was bitten at multiple sites by a rabid dog and received the first postexposure prophylaxis with Pasteur's vaccine. Remarkably, Joseph survived (Koprowski, 1985 cited in Lyles and Rupprecht, 2007). Each of the other currently licensed live vaccine viruses has a relatively clear lineage (Graham and Crowe, 2007).
Pasteur's vaccine, with all its modifications, became the accepted rabies prophylactic throughout the world in the early 20th century. Thus, he started the new field of medicine called immunology. Pasteur also became famous for his concept of the 'germ theory'. This is still the theory modern medicine uses to explain all illness. Thus we have created a 'war on bugs' that we seem to be losing (O'Driscoll, 2008). It is interesting to note that on his deathbed Pasteur recanted his prior work of blaming the microorganism. His last words were "seed is nothing, soil is everything". In Chinese medicine it is said that "it is not the agent, but the terrain". Both are saying the same thing - the germ is nothing, but the host's resistance is everything. These concepts lay the foundation for all forms of holistic medicine (O'Driscoll, 2008).

Postexposure prophylaxis against rabies through simultaneous administration of antirabies serum and vaccine was introduced in 1889 by Babes (1912 cited in Lyles and Rupprecht, 2007). This approach found few adherents and languished until about 1940, when interest in the use of serum containing rabies virus antibodies in India (Rao et al., 2004), was revived. In a trial organized by the World Health Organization (WHO) in 1954, the combined use of serum and vaccine was found to be more protective than vaccine alone, an observation later corroborated by Chinese findings. Each of the other currently licensed live vaccine viruses has a relatively clear lineage (Graham and Crowe, 2007). In the 1960s, a rabies virus grown in human diploid cells was used to produce a safe and efficacious vaccine, eliminating many of the problems connected with vaccines produced in brain tissue (Lyles and Rupprecht, 2007). In 1961, recommendations were made that a serum analysis of the blood was the best way to determine the immunological protection of vaccines. Historically (1957-1980s), several major modern human rabies vaccines include Duck Embryo Vaccine (DEV), which was commercialized in 1957; Human Diploid Cell Vaccine (HDCV) was introduced in 1978; Purified Chicken Embryo Cell Vaccine (PCECV) was developed in 1984; and a Purified Vero Cell Rabies Vaccine (PVRV) was developed in the late 1980s (Wu et al., 2009).

Before the 1980s, nerve tissue-derived Semple vaccine (NTV) was manufactured using the fixed RABV Beijing strain 3aG, which was isolated in 1931 (Wu et al., 2009). The development of safe and effective rabies virus vaccines applied in attractive baits resulted in the first field trials in Switzerland in 1978. Thereafter, technical improvements occurred in vaccine quality and production, including the design of recombinant viruses, as well as in the ease of mass distribution of millions of edible baits over large geographical areas (Rupprecht et al., 2004). Also, according to Wu et al. (2009), after the 1980s, Primary Hamster Kidney Cells (PHKC) rabies vaccine using the same 3aG strain was investigated as a substitute for NTVs. The heteroploid VERO cell line was introduced in 1982 to the production of inactivated rabies vaccine; it retained all the advantages of the human diploid cell system, while offering the possibility of the large-scale industrial production of PVRV. In 1988, cell culture rabies vaccines for human use, highly immunogenic and well tolerated, were now used for pre-exposure immunization as well as for post-exposure treatment. In 1992, WHO recommends a new inactivated rabies vaccine grown on Vero cells, (PVRV) with the vaccine cultivated on Human Diploid Cells Vaccine (HDCV), for both pre and post-exposure prophylaxis (Nagarajan et al., 2008). Following the widespread use of HDCV and PVRV, many comparative clinical trials have been conducted, studying the safety and predictive values of these two vaccines. In 2001, WHO issued a resolution for the complete replacement of NTVs by 2006 with cell-culture rabies vaccines (Wu et al., 2009).

The viral vaccines used in the past have included live attenuated vaccines, killed virus vaccines, and subunit vaccines. Both the killed virus vaccine and the recombinant subunit vaccine were new to the modern era of virology (Levine and Enquist, 2007). New vaccine technologies now include the use alone or in combination of attenuated viral vectors such as vaccinia virus or adenovirus and DNA plasmids that express viral proteins from strong promoters. So-called therapeutic vaccines will boost the immune systems of individuals using specific cytokines or hormones in combination with new adjuvants to stimulate immunity at specific locations in the host or to tailor the production of immune effector cells and antibodies (Levine and Enquist, 2007; Morrow and Weiner, 2008). Considering that the first vaccines (for smallpox) were reported in the Chinese literature of the 10th century (Fenner and Nakano, 1988), these ideas can be traced back in time to the origins of virology (Levine and Enquist, 2007). In recent years, the combination of serum and vaccine is the recommended standard for prophylaxis in human rabies exposure. This vaccine and others are used widely throughout the world, although for economic reasons, several developing countries still use nervous tissue vaccines (Lyles and Rupprecht, 2007). Today purified and concentrated Vero cell rabies vaccines using the 3aG and CTN-1 strains have been developed. The PVRV, using a RABV purified Vero (PV) strain imported from the US Centers for Disease Control and Prevention (CDC), is also being developed to meet the increasing demand for human rabies vaccine (Wu et al., 2009). According to Wu et al. (2009), from 1885, when the first human rabies vaccination occurred, to 1994, when the rabies vaccine, Street-Alabama-Dufferin (SAD) B19 strain was engineered with reverse genetics, methods for RABV manipulation have changed fundamentally from random attenuation to defined modifications. However, the basic concept for rabies vaccine development has not changed for more than a century.
Generations of vaccines production and the present status of rabies vaccine development:

First vaccine revolution: Pasteur developed rabies vaccine. It was the first attenuated viral vaccine that passed through nerve cords of rabbits. Although relatively effective, ‘killed’ vaccines are sometimes not as effective at preventing infection as ‘live’ virus vaccines, often because they fail to stimulate protective mucosal and cell-mediated immunity to the same extent. A more recent concern is that these vaccines contain virus nucleic acids, which may themselves be a source of infection, either of their own accord (e.g., (+) sense RNA virus genomes) or after recombination with other viruses (Alan, 2005).

Second vaccine revolution: There are three basic types of vaccines under this category: subunit vaccines, inactivated vaccines, and live-virus vaccines. Subunit vaccines consist of only some components of the virus, sufficient to induce a protective immune response but not enough to allow any danger of infection. In general terms, they are completely safe, except for very rare cases in which adverse immune reactions may occur. Unfortunately, at present, they are also the least effective and most expensive type of vaccines. There are several categories of such vaccines: synthetic, recombinant, and virus vectors. Synthetic vaccines are short, chemically synthesized peptides. None is currently in use (Alan, 2005). Most vaccines in current use are inactivated (Hunt, 2000). Inactivated vaccines are produced by exposing the virus to a denaturing agent under precisely controlled conditions. The objective is to cause loss of virus infectivity without loss of antigenicity. Obviously, this involves a delicate balance; however, inactivated vaccines have certain advantages, such as generally being effective immunogens (if properly inactivated), being relatively stable, and carrying little or no risk of vaccine-associated virus infection (if properly inactivated, but accidents can and do occur) (Alan, 2005). Live (attenuated) virus vaccines strategy relies on the use of viruses with reduced pathogenicity to stimulate an immune response without causing disease. The vaccine strain may be a naturally occurring virus (e.g., the use of cowpox virus by Jenner) or artificially attenuated in vitro (e.g., the oral poliomyelitis vaccines produced by Albert Sabin). The advantage of attenuated vaccines is that they are good immunogens and induce long-lived, appropriate immunity (Hunt, 2000; Alan, 2005).

Third vaccine revolution: Recombinant vaccines are produced by genetic engineering. Such vaccines have been already produced and are better than synthetic vaccines because they tend to give rise to a more effective immune response. Virus vectors are recombinant virus genomes genetically manipulated to express protective antigens from (unrelated) pathogenic viruses. The idea here is to utilize the genome of a well-understood, attenuated virus to express and present antigens to the immune system (Alan, 2005). Many different viruses offer possibilities for this type of approach, but the most highly developed system so far is based on the vaccinia virus (VV) genome. VV-rabies recombinants have been used to eradicate rabies in European fox populations (Alan, 2005). DNA vaccines and RNA interference (RNAi) are the newest type of vaccine and consist of only a DNA molecule encoding the antigen(s) of interest and, possibly, costimulatory molecules such as cytokines. The deliberate introduction of a DNA plasmid carrying a protein-coding gene that transfects cells in vivo at very low efficiency and expresses an antigen that causes an immune response. These are often called DNA vaccines but would better be called DNA-mediated or DNA-based immunization since it is not the purpose to raise antibodies against the DNA molecules themselves. All of the above means that DNA vaccines are cheap and therefore likely to be developed against pathogens of lesser economic importance (at least to drug companies) (Hunt, 2000; Kutzler and Weiner, 2004).

The concept behind these vaccines is that the DNA component will be expressed in vivo, creating small amounts of antigenic protein that serves to prime the immune response so that a protective response can be rapidly generated when the real antigen is encountered. In theory, these vaccines could be manufactured quickly and should efficiently induce both humoral and cell mediated immunity. Initial clinical studies have indicated that there is still some way to go until this experimental technology becomes a practical proposition (Kutzler and Weiner, 2004; Alan, 2005). It has been discovered that a relatively newly discovered phenomenon known as RNAi can be used to inhibit virus replication of a wide variety of viruses in vitro. REMINISCENT of the triggering of interferons, dsRNA serves as a trigger for RNAi. An enzyme highly conserved in eukaryotic evolution, called Dicer, cleaves dsRNA into 21- to 23-nt-long fragments known as small inhibitory RNAs (siRNAs), which ultimately result in destruction of the homologous target RNA. In some species, but apparently not in humans, siRNA can also direct the synthesis of more dsRNA, leading to amplification of the effect and allowing the effect of RNAi to spread from cell to cell (Alan, 2005).

New generations of rabies vaccines: Rabies virus is considered as a unique virus, 5 groups of rabies fixed strains are used throughout the world to produce human rabies vaccines: Pasteur, Beijing, Flury, Fuenzalida and SAD strains. The different rabies vaccines should be classified according to the cell system used to cultivate the virus: Animal systems are still employed to produce the old traditional vaccines-Semple and SMB—which continue to be produced in several countries; Primary cell
systems, particularly Hamster Kidney and Chick embryo cells, are used (Nagarajan et al., 2008). The Pasteur-derived strains, designated PV or PM, are the most widely used for the production of traditional vaccines of the Semple or Suckling Mouse Brain (SMB) types, but also for the production of modern cell culture vaccines: Human Diploid and Purified Vero Cell Vaccines (HDCV and PVRV) (Nagarajan et al., 2008). However, new generation of low-cost, purified rabies vaccines released in the last 2 years promises a revolution in rabbies immunization in less-developed, rabies endemic countries, providing protection comparable to that of human diploid cell vaccine at the cost of Semple-type vaccine. The principal human rabies vaccines produced worldwide at present using cell culture are compared, in terms of their technical characteristics and their capacity economically to face worldwide vaccine needs. Cell culture rabies vaccines have become widely available in developing countries, virtually replacing the inferior and unsafe nerve tissue vaccines (Nagarajan et al., 2008). Today, the greatest needs are in tropical countries, where only a limited amount of modern cell culture vaccines are used.

**Human rabies vaccines:** Major human rabies vaccines include NTV, PHKCV, DEV, HDCV, PCECV, and PVRV (Wu et al., 2009). Since WHO had already strongly recommends the replacement of NTVs with modern vaccines, extensive clinical experience supports the use of these vaccines for intramuscular and intradermal pre- and post-exposure prophylaxis, including in special situations (Toovey, 2007). Cell culture rabies vaccines have become widely available in developing countries, virtually replacing the inferior and unsafe nerve tissue vaccines (Nagarajan et al., 2008). For both HDCV and PVRV, production security is guaranteed by the existence of a master cell seed and working cell bank, with a complete history of the cell, a limited number of passages and permanent and total quality control of the cell substrate (Nagarajan et al., 2008). A year-long protection could easily be provided with the both vaccines, till to the 1st booster dose administration. For both HDCV and PVRV, production security is guaranteed by the existence of a master cell seed and working cell bank, with a complete history of the cell, a limited number of passages and permanent and total quality control of the cell substrate (Nagarajan et al., 2008).

**Human diploid cell vaccine (HDCV):** HDCV is currently considered the gold standard. No serious side effects had occurred with HDCV, although some vaccinees complaining of redness, induration or local pain and exceptionally of fever and lymphadenopathy have been reported. A year-long protection could easily be provided with the vaccine, till to the 1st booster dose administration. The use of the human diploid cell system permitted the development of the HDCV, the most widely distributed cell culture rabies vaccine, and today considered as the reference vaccine (Nagarajan et al., 2008).

**Purified Vero cell rabies vaccine (PVRV):** Also, no serious side-effects had occurred with PVRV. Some vaccinees complaining of redness, induration or local pain and exceptionally of fever and lymphadenopathy have also been reported (Nagarajan et al., 2008).

**Purified chick embryo cell (PCEC) vaccine (Rabipur):** Several pre- and post-exposure controlled vaccine trials and clinical studies have shown that the PCEC vaccine, Rabipur, is as safe and effective as the HDCV, which is currently considered the gold standard. Additionally, PCEC vaccine does not result in immune-mediated hypersensitivity reactions following booster doses seen in about 6% of those receiving HDCV boosters following an initial series of HDCV.

**Purified rabies vaccine cultured on Vero cells (Verorab, sanofi pasteur):** Verorab, sanofi pasteur is WHO-approved for pre- and post-exposure prophylaxis by intradermal and intramuscular routes as an effective and inexpensive option for developing countries. During 20 years of use, over 40 million doses of Verorab have been administered in more than 100 countries. No serious adverse event due to Verorab has been reported in clinical trials. Verorab is not associated with post-boosting serum sickness. Extensive clinical experience supports the use of Verorab for intramuscular and intradermal pre- and post-exposure prophylaxis, including in special situations (Toovey, 2007).

**Animal rabies vaccines:** In contrast to human rabies vaccine development, animal rabies vaccine development in most countries has not progressed. In the United States alone, 11 different rabies vaccines are licensed for dogs, 12 for cats, 1 for ferrets, 3 for horses, 4 for cattle, and 5 for sheep (Briggs et al., 2007). However, in places like China, only 1 pentavalent vaccine is licensed, and 1 Flury-Low Egg Passage (LEP) vaccine for dogs has been tentatively approved and no regional RABV isolates were characterized for animal vaccine development (Wu et al., 2009). Currently, High Egg Passage (HEP), LEP, Evelyn–Rokitnicki–Abelseth (ERA), PV, and Challenge Virus Standard (CVS) strains being developed as vaccine candidates originate from other countries and have an unclear biological background. Consequently, development of animal rabies vaccines using carefully characterized RABV strains should be prioritized as a fundamental task (Wu et al., 2009).
**Vaccinia rabies glycoprotein (V-RG):** V-RG vaccine was the first recombinant rabies vaccine to be constructed, field tested, and considered for regulation in Europe and North America for wildlife rabies control. This vaccine has been extensively reviewed to ensure safety (tested in >40 species of mammals and birds) and efficacy (proved against severe rabies challenge in target species). Following the success of the V-RG vaccine against fox rabies in Belgium and France, preliminary field trials suggest its potential utility for rabies control in raccoons, foxes, and coyotes in the U.S. (Lyles and Rupprecht, 2007).

**Street-Alabama-Dufferin (SAD) B19:** SAD B19 is an attenuated vaccine virus for oral vaccination of carnivores against rabies. The safety of SAD B19 has been investigated by Vos et al. (1999) who concluded that it is suitable for oral vaccination campaigns for carnivores against rabies. The use of the techniques and strategies of oral immunization of foxes against raccoons using SAD B19 can eliminate wildlife rabies among foxes and raccoon dogs, as European experience has shown. The disease then also disappears completely in domestic animals and man.

**Vectored vaccine:** An oral baculovirus-vectored vaccine induces protective immunity in raccoons. Other orthopoxviruses have been considered as vectors of lyssavirus antigens, but these have not yet been field-tested (Lyles and Rupprecht, 2007).

**Novel approaches and future trend in vaccine production:** Some believe that vaccine production should reflect the design of matched field isolates for regional control (Wu et al., 2009). However, vaccine-matching investigations to address concerns about mismatch between vaccine strains and epidemic RABV isolates are redundant (Dong et al., 2007). All fixed RABV strains recommended by WHO, such as PV, CVS, LEP, HEP, ERA, and SAD variants, have been successfully used in industrialized countries, where rabies is well controlled. Any potent rabies vaccine will protect against raccoons (Wu et al., 2009). The currently available modified-live rabies virus vaccines have either safety problems or do not induce sufficient protective immunity in particular wildlife species. Therefore, there is a need for the development of new live rabies virus vaccines that are very safe and highly effective in particular wildlife species. Meanwhile, new types of vaccines are being developed by applying gene manipulation techniques to rabies virus in order to overcome the disadvantages of current vaccines (Sugiyama and Ito, 2007). The progress made in the development of new-generation rabies vaccines with the goal of elimination or control of rabies in world include:

**Genetically modified, reverse-engineered live attenuated rabies vaccines:** In a report by Morimoto et al. (2001), a novel approach to the development of rabies vaccines using genetically modified, reverse-engineered live attenuated rabies viruses was described. This approach entails the engineering of vaccine rabies virus containing G proteins from virulent strains and modification of the G protein to further reduce pathogenicity. Strategies employed included exchange of the arginine at position 333 for glutamine and modification of the cytoplasmic domain. According to Morimoto et al. (2001), the ability to confer protective immunity depended largely upon conservation of the G protein antigenic structure between the vaccine and challenge virus, as well as on the route of immunization.

**Reverse genetics approach:** Based on previous observations indicating that the potency of a vaccine is significantly increased if the G protein of the vaccine strain is identical to that of the target virus, Dietzschold and Schnell (2002) have used a reverse genetics approach to engineer viruses that contain G proteins from virus strains associated with relevant wildlife species. Furthermore, because recent data also indicate that the pathogenicity of a particular rabies virus strain is inversely proportional to its ability to induce apoptosis and that low-level apoptosis-inducing ability is associated with low anti-viral immune responses, Dietzschold and Schnell (2002) inserted genes encoding pro-apoptotic proteins to stimulate immunity or otherwise interfere with viral pathogenesis into these recombinant viruses to enhance their efficacy and safety. The application of Reverse genetics technology in has also been applied in the study of Rabies Virus (RV) pathogenesis and for the development of novel RV vaccines (Schnell et al., 2005).

**The RABV G protein gene:** The RABV G protein gene has also been inserted into the human adenovirus genome. Preliminary experiments have shown adequate G protein expression in cell culture and efficacy in several immunization trials in captive animals. Newer constructs (e.g., RABV glycoprotein inserts using the adenovirus E1 region, incapable of replication in host tissue) are also under consideration in a variety of species. A recombinant plasmid pTargetT.rabgp containing the glycoprotein (G) gene of Rabies Virus (RV) was constructed and produced for immunogenicity studies on mice and dogs by Rai et al. (2005). Their results indicated that the pTargetT.rabgp plasmid could be used as a rabies DNA vaccine.

**Monoclonal antibody-based human rabies immunoglobulin (RIG):** Limitations inherent to the conventional RIG of either equine or human origin have prompted scientists to look for monoclonal antibody-
based human RIG as an alternative. Fully human monoclonal antibodies have been found to be safer and equally efficacious than conventional RIG when tested in mice and hamsters (Nagarajan et al., 2008). These novel human monoclonal antibodies, their production, and the significance of plants as expression platforms were emphasized.

**Considerations for rabies vaccines and vaccinations in global rabies control and possible eradication:** In keeping pace with various vaccination recommendations and considerations, people at high risk of exposure to rabies, such as veterinarians, animal handlers, rabies laboratory workers, spelunkers, and rabies biologics production workers should be offered rabies vaccine. The vaccine should also be considered for: (1) people whose activities bring them into frequent contact with rabies virus or with possibly rabid animals, and (2) international travelers who are likely to come in contact with animals in parts of the world where rabies is common (CDC, 2006). However, this is a risky business; if protection of the population falls below a critical level, epidemics can easily occur.

**Designing effective vaccines:** According to Alan (2005), to design effective vaccines, it is important to understand both the immune response to virus infection and the stages of virus replication that are appropriate targets for immune intervention. To be effective, vaccines must stimulate as many of the body’s defence mechanisms as possible. In practice, this usually means trying to mimic the disease without, of course, causing pathogenesis—for example, the use of nasally administered influenza vaccines and orally administered poliovirus vaccines. To be effective, it is not necessary to get 100% uptake of vaccine. ‘Herd immunity’ results from the break in transmission of a virus that occurs when a sufficiently high proportion of a population has been vaccinated (Alan, 2005).

**Types and sorts of rabies vaccines:** Cell or tissue culture vaccines e.g., Nobivac Rabies (Intervet), Rabisin (Merz), Verorab (Sanofi pasteur) have been used extensively. Purified rabies vaccine cultured on Vero cells (Verorab, sanofi pasteur) is WHO-approved for pre- and post-exposure prophylaxis by intradermal and intramuscular routes. Nerve tissue origin vaccines, although used extensively in some parts of the world, are not recommended if cell or tissue culture vaccines are available (Toovey, 2007).

**Doses and route of vaccination:** The number of doses and route of vaccination with a tissue culture or cell culture origin vaccine differ in various regions of the world. The administration of a RIG is generally recommended in conjunction with the first dose of the rabies vaccine. For instance, pre-exposure prophylaxis using Verorab is confirmed immunogenic in 1437 subjects by all routes, with prompt responses following boosting; Verorab boosts effectively in subjects pre-immunized with HDCV (Toovey, 2007).

**Safety and efficacy of rabies vaccines:** Vaccine safety is a major concern for patients, parents, healthcare providers, public health officials and vaccine manufacturers (Loughlin et al., 2009). Doubt has sometimes been cast upon the protective effect of rabies antibodies in serum. Several studies of the safety of rabies PEP for pregnant patients demonstrated no association between treatment and adverse outcomes (Abazeed and Cinti, 2007). Figueroa et al. (1994) reported 2 patients with rabies exposure during the second and third trimester of pregnancy that received immunization respectively with Vero cell vaccine and Fuenzalida vaccine. A study by Sudarshan et al. (2007) on 14 pregnant women who received rabies PEP showed that none had any adverse events to either vaccine or equine RIG. In one study by Abazeed and Cinti (2007), tissue culture-derived vaccines and HIG did not lead to an increased risk for congenital anomalies. Although these studies are not comprehensive in their assessment of all reproductive outcomes, they do suggest that PEP is generally safe. Manickama et al. (2008) study on the efficacy of two commercially available rabies vaccines and a 5- or 3-dose vaccination regime indicated that none of the vaccinated dogs showed any clinical signs of rabies at any stage. All of their brain tissue samples taken at the end of the study were found negative for rabies viral antigen. Both vaccines were found to be safe and effective in preventing rabies when inoculated intramuscularly applying the 5-dose regime (0, 3, 7, 14 and 28 days). WHO recommended PEP for rabies using PCEC-K is also considered effective and safe (Yanagisawa et al., 2008). Modern PEP is a combination of active and passive immunization, with 100% efficacy in rabies prevention if the process strictly adheres to WHO recommended guidelines (Rupprecht et al., 2006; Manning et al., 2008). After rabies exposure in humans, PEP is initiated or withheld based on the postmortem diagnosis of animals that were the source of exposure. Because human rabies vaccines are produced in cell culture using modern technology, the vaccine quality should follow the standard recommended by WHO (Wu et al., 2009).

**Potency of rabies vaccines:** A key factor for the success of the dog rabies control program in Mexico was the supply of potent canine rabies vaccines (Lucas et al., 2008). The minimum potency for all cell-culture and purified embryonated egg rabies vaccines is 2.5 IU per intramuscular dose using the National Institutes of Health test (WHO, 2005). Potency and efficacy of rabies vaccines determined by Minke et al. (2009) by comparing...
antibody responses after vaccination of 30 laboratory dogs with two inactivated rabies vaccines (RABISIN, Merial France) and NOBIVAC, Intervet International) performed differently in terms of magnitude and persistence of rabies antibodies titers. RABISIN induces higher and sustained antibody titres against rabies, increasing the flexibility for the time of blood sampling after primo-vaccination.

**Testing for potency, safety, effectiveness and efficacy of rabies vaccines:** The need to verify the effectiveness of rabies vaccination has become widespread, particularly in the context of international trading of domestic carnivores from infected to rabies-free territories. In compliance with regulatory guidelines, immunogenicity as well as safety of adjuvanted candidate vaccines should be evaluated preclinically and clinically (Garçon et al., 2006; Garçon, 2009). Preclinical safety analyses of the candidate vaccine (product characterization, lot-release testing, elucidation of the mode of action and toxicology) are part of preclinical safety packages on which clinical development plans and safety evaluations are based (Verstraeten et al., 2008; Garçon, 2009). Safety monitoring following introduction of new vaccines includes assessment of potential new onset Autoimmune Diseases (AID). Interpreting the significance of these cases is difficult because knowledge about AID background incidence rates is limited (Klein et al., 2009). Causality assessment can be based on modified WHO criteria that classify the causal relationship between the vaccine and the adverse event (WHO, 2001; Loughlin et al., 2009). In term of vaccine efficacy, serological tests for rabies are also often used by laboratories in infected territories to assess the efficacy of campaigns aimed at the eradication of the disease via oral vaccination of wildlife. The adaptation of these methods should provide the means to titrate specific antibodies in dogs during mass parenteral vaccination in countries infected by canine rabies (Servat et al., 2006).

In term of effectiveness of vaccines, serology remains the only way to monitor the effectiveness and immunological protection of vaccination of humans and animals against rabies (Servat et al., 2006). Though a simple test, but there has been some controversy as to what titer level will provide protection from the clinical disease, and what level tells if there has been exposure to the disease (O’Driscoll, 2008). Many techniques for determining the level of rabies antibodies have been described and this include seroneutralisation techniques (such as tests for Fluorescent Antibody Virus Neutralization, FAVN and Rapid Fluorescent Focus Inhibition, RFFIT), Enzyme-Linked Immunosorbent Assay (ELISA), and in-vivo tests (the mouse neutralisation test, MNT). The standardization of serological techniques, approval of laboratories and proficiency tests are key concepts to ensure the practicability of such systems (Servat et al., 2006). The next way is to believe the biologics companies. This may not be an ideal choice since most animal researchers and clinicians would vaccinate animals and they would break out with the disease many years ago. There have also been cases were perfectly healthy animals coming down with the diseases they were being vaccinated against (O’Driscoll, 2008).

**Challenges and Concerns Facing Current Rabies Vaccines Approaches:** The reemergence of rabies in China raises concerns. The high incidence of rabies reported in this reemergence leads to numerous concerns which include; a potential carrier-dog phenomenon, undocumented transmission of rabies virus from wildlife to dogs, counterfeit vaccines, vaccine mismatching, and seroconversion testing in patients after their completion of postexposure prophylaxis (PEP). These concerns are all scientifically arguable given a modern understanding of rabies (Wu et al., 2009). Rabies virus is not a single entity but consists of a wide array of variants that are each associated with different host species. These viruses differ greatly in the antigenic makeup of their G proteins, the primary determinant of pathogenicity and major inducer of protective immunity. Due to this diversity, existing rabies vaccines have largely been targeted to individual animal species (Morimoto et al., 2001). The prevalent vaccine used in most developing countries is still the NTV, which, although improved, necessitates the use of long and painful application schedules and fails to provide safe and reaction-free protection. However, cell lines are presently the most interesting approach for vaccine production. Today, major technical problems were associated with subunit vaccines, this include their relatively poor antigenicity and the need for new delivery systems, such as improved carriers and adjuvants. Their disadvantages is that they are not usually very effective immunogens and very costly to produce; however, because they can be made to order for any desired sequence, they have great potential for the future (Hunt, 2000; Alan, 2005).

**Inactivation:** Problems remained with Pasteur vaccine, however, because improperly inactivated virus caused rabies, and animal brain tissue induced allergic reactions leading to neuroparalytic accidents. Moreover, perhaps most importantly, the vaccine was not very effective in cases of severe bites, such as those inflicted on the face and neck by rabid wolves and dogs (Lyles and Rupprecht, 2007). The challenges and concerns associated with inactivated vaccines is their disadvantage. It is not possible to produced inactivated vaccines for all viruses, as denaturation of virus proteins may lead to loss of antigenicity (Alan, 2005). Despite these difficulties, vaccination against virus infection has been one of the...
great triumphs of medicine during the twentieth century. Although prevention of infection by prophylactic vaccination is much the preferred option, postexposure therapeutic vaccines can be of great value in modifying the course of some virus infections. This include rabies virus, where the course of infection may be very long and there is time for postexposure vaccination to generate an effective immune response and prevent the virus from carrying out the secondary replication in the CNS that is responsible for the pathogenesis of rabies (Alan, 2005).

**Attenuated:** Set against the advantages of live (attenuated) virus vaccines are their many disadvantages. They are often biochemically and genetically unstable and may either lose infectivity (becoming worthless) or revert to virulence unexpectedly. Another major challenge facing attenuated vaccines is that despite intensive study, it is not possible to produce an attenuated vaccine to order, and there appears to be no general mechanism by which different viruses can be reliably and safely attenuated. Contamination of the vaccine stock with other, possibly pathogenic viruses is also possible—this was the way in which SV40 was first discovered in oral poliovirus vaccine in 1960. Inappropriate use of live virus vaccines, for example, in immunocompromised hosts or during pregnancy may lead to vaccine-associated disease, whereas the same vaccine given to a healthy individual may be perfectly safe (Hunt, 2000; Alan, 2005).

**Recombinant DNA technology:** Third generation of vaccines also faces similar challenges, for instance, recombinant vaccines are difficult to produce and no human example is clearly successful yet, although many different trials are currently underway (Alan, 2005). Enhancing DNA vaccine potency remains also a challenge (Hung et al., 2006; Muthumani et al., 2009). According to McRearden (2008) and Blanco (2008), “One of the major concerns with these methods is the unpredictability and interaction of the final vaccine product with the proteins or DNA of the host. A document from the FDA states: Genetic toxicity: Integration of the plasmid DNA vaccine into the genome of the vaccinated subjects is an important theoretical risk to consider in preclinical studies. The concern is that an integrated vaccine may result in insertional mutagenesis through the activation of oncogenes or inactivation of tumor suppressor genes. In addition, an integrated plasmid DNA vaccine may result in chromosomal instability through the induction of chromosomal breaks or rearrangements.

Another group advises that research findings in gene therapy and vaccine development show that naked/free nucleic acids constructs are readily taken up by the cells of all species including human beings. These nucleic acid constructs can become integrated into the cell's genome and such integration may result in harmful biological effects, including cancers”. Also, the technical problems currently associated with RNAi vaccines are in their delivery into cells in the body. If this can be overcome, RNAi could become an important new tool in treatment and prevention of virus diseases (Alan, 2005). And to reiterate the danger of tumorigenic cell lines, some researchers said more recently, recombinant DNA technology has expanded beyond bacterial cells to mammalian cells, some of which may also be tumorigenic (McRearden, 2008; Blanco, 2008). Other major challenges and concerns facing current approaches of rabies vaccines include: misconceptions about vaccines, annual pet vaccination, and lack of standard procedures for assessing vaccine safety and efficacy and adverse effects after vaccination.

**Misconceptions about vaccines:** Vaccines are the basis of the medical and veterinary medical future, the belief being that, if a vaccine can be made to every disease, then all disease can be prevented. This presupposes that 1) disease attacks from outside and has nothing whatsoever to do with the person or animal themselves; 2) that vaccines actually always protect 100%; and 3) that vaccines themselves are only beneficial and cannot cause harm. None of these are true (O’Driscoll, 2008). There is a growing list of research into and information on the problems that can be caused by vaccines (O’Driscoll, 2008; McRearden, 2008; Blanco, 2008).

**Annual pet vaccination against rabies:** There are lack of scientific evidence to support our current practices of vaccination in animals. According to Schultz and Philips (1992 cited in O’Driscoll, 2008), “A practice that was started many years ago, that lacks scientific validity or verification is annual vaccinations. Almost without exception there is no immunologic requirement for annual revaccination. Immunity to viruses persists for years or for the life of the animal. Furthermore, revaccination with most viral vaccines fails to stimulate an anamnestic response as a result of interference by existing antibodies (similar to maternal antibody interference). The practice of annual vaccination in our opinion should be considered of questionable efficacy unless it is used as a mechanism to provide an annual physical exam or is required by law (i.e., certain states require annual revaccination for rabies).” According to O’Driscoll (2008), there has been much debate on the subject of annual pet vaccination, chiefly in response to concerns voiced by pet owners. The veterinary profession is largely unaware of the range of side-effects vaccines can stimulate, and consequently they go unreported. It is acknowledged that certain individuals are genetically predisposed to suffer adverse reactions to vaccines. On the whole, the veterinary profession in the UK has defended annual vaccination, on the basis of information largely supplied by the pharmaceutical
industry. Yet there are wider issues to consider. A radical rethink of the vaccination programme is necessary - immunisation programmes need not be abandoned, but reassessed. The first subject for assessment is whether all animals should be vaccinated, irrespective of their genetic status (O’Driscoll, 2008; McRearden, 2008; Blanco, 2008).

**Lack of standardized procedure:** In most cases of vaccine efficacy assessment, recommended serological tests are carried without any standardized procedure. According to Servat et al. (2006), on the basis of past experiences reported in rabies serology and its harmonization throughout laboratories worldwide, most researchers propose an adapted standard technique for the serological monitoring for rabies in wildlife at the European level. Such harmonization would allow the monitoring of vaccination campaigns to be enhanced by increasing the exchange of epidemiological data, with the ultimate goal being the eradication of rabies in Europe and in the world.

**Adverse effects following vaccination:** In the past, some researchers faced with proven risks of rabies infection have vaccinated free-ranging wild dogs. However, the death from rabies of some of the vaccinated animals has led several authors to question the value of rabies vaccination as a tool in wild dog management (Ginsberg and Woodroffe, 1997). There are clinical manifestations and harmful effects of the use and abuse of vaccinations. Unfortunately, adverse reactions to vaccines have been considered to be the immediate hypersensitivity reactions of anaphylaxis. This severely limits the types of reactions that are ever even considered to be related to vaccines (O’Driscoll, 2008; McRearden, 2008; Blanco, 2008). Other problems surface which make accurate tallying of adverse reactions difficult. Some authors have therefore claimed that the vaccines provide protection by keeping the body in a diseased state of health. When a perfectly healthy individual is given viruses that cause illness, the animal is going to manifest illness-related symptoms. This healthy individual is asked to maintain a low-level stimulation state of rabies. According to these authors (O’Driscoll, 2008; McRearden, 2008; Blanco, 2008), often the animal will not manifest the illness it is vaccinated for, at least not in its acute form, but it will manifest in other conditions. Usually these conditions are inherited weaknesses. However, the adverse effects associated to vaccinations include:

**Vaccinosis:** Vaccinosis is the reaction from common inoculations (vaccines) against the body's immune system and general well being. These reactions might take months or years to show up and will cause undue harm to future generations. Vaccinosis, which is used to describe the chronic illness that results from vaccination, should be understood as the disturbance of the vital force by vaccination that results in mental, emotional and physical changes that can, in some cases be a permanent condition (McRearden, 2008, Blanco, 2008).

**Chronic symptoms after vaccination:** Chronic symptoms look very much like the acute illnesses but they are often not life-threatening unless allowed to continue for years and years. According to O’Driscoll (2008), McRearden (2008) and Blanco (2008), for rabies we often see: restless nature (suspicion of others, aggression to animals and people); changes in behavior (aloofness, unaffectionate, desire to roam, OR clinging, separation anxiety, ‘velcro dog’, voice changes e.g., hoarseness and excessive barking, chronic poor appetite, very finicky, eating wood, stones, earth and stool); paralysis of throat or tongue, sloppy eaters and drooling; dry eye (loss of sight and cataract); destructive behavior (shredding bedding, seizures, epilepsy, twitching, increased sexual desire, sexual aggression, irregular pulse, heart failure and reverse sneezing) and restraining can lead to violent behavior and self-injury self-mutilation (tail chewing).

**Familial illness following vaccination:** Some of the familial illnesses include any auto-immune disease (such as lupus, red cell aplasia, auto-immune hemolytic anemia, cardiomyopathies); neoplasias (such as fibrosarcomas, mast cell tumors, thyroid tumors, etc.); inflammatory bowel disease, eczematous ears, any dermatological condition, warts, lipomas, poor hair coats, stomatitis, periodontal disease, thyroid disease, and the list goes on and on (O’Driscoll, 2008; McRearden, 2008; Blanco, 2008).

**Anaphylactic shock:** Anaphylactic shock is the most well-known consequence, but other consequences are possible, namely types I, II, III and IV hypersensitivity reactions, which include tissue injury, arthritis, lupus, and kidney and liver damage. Vaccines sensitize genetically susceptible organisms. Animals from families that are known to suffer allergic/inflammatory conditions are at most risk from live vaccines. Frick and Brooks, in 1983, demonstrated that dogs predisposed to develop atopic dermatitis did not do so if vaccinated after being exposed to an allergen, but did develop the condition when they were vaccinated first and then exposed to an allergen (McRearden, 2008; Blanco, 2008).

**T cell immunodeficiencies:** Vaccines are also acknowledged to cause T cell immunodeficiencies (Blanco, 2008). However, it should be borne in mind that serum reactions are known to develop sometime after vaccine administration, and that some vaccine components are not easily metabolized, remaining in the system for some considerable time.
Generically stimulations of the body: As it has been demonstrated, that a wide range of human vaccines can stimulate arthritis, it makes sense to consider, also, that animal vaccines might also stimulate this condition (O’Driscoll, 2008). Glickman and GogenEsch (1997) showed that vaccinated group developed significant levels of autoantibodies against fibronectin, laminin, DNA, albumin, Cytochrome C, transferring, cardiolipin and collagen in a study on the effects of routinely used vaccination protocol of commercial multivalent vaccine and rabies vaccine on the immune and endocrine system of Beagles. According to O’Driscoll (2008), this is the fundamental problem with vaccines: they are generically stimulating to the body, usually creating illness where there once was none. Some researchers have suggested that something in the vaccine could be one of the etiologies (in the genetically susceptible dog) of such diseases as cardiomyopathy, lupus, erythematosus, glomerulonephritis, etc. However, other factors other than generic reaction are also responsible for adverse reaction after vaccination.

Causes of vaccine associated problems: A vaccine, like any medicine, is capable of causing serious problems, such as severe allergic reactions. The risk of a vaccine causing serious harm, or death, is extremely small. Serious problems from rabies vaccine are very rare (CDC, 2006). The causes of vaccine-associated problems include factors that are linked to vaccine, vaccinations, nutrition and environment.

Vaccine factors:
Type of vaccines: Vaccines usually have numerous viruses as well as other ingredients in them. Exceptions to this include the rabies, corona, and bordatella vaccines. Herein lays the first and second problem with the vaccination process. The process of injecting numerous viruses at one time into the body does not mimic in any way what we would see in the natural world. There would never be such an enormous exposure to that many microorganisms at one time. These diseases have never, in the real world, occurred at one time, never (Glickman, 2000).

Chemical agent used: Typically, the chemical agent used to alter the virus is formalin or formaldehyde, a known carcinogen. Attenuating the virus so that it cannot attach to a cell wall and infect that cell is a good idea, but not all the virus particles may be altered. Some may escape attenuation and are free to cause disease. This may be part of the reason that why ‘breaks’ are seen in vaccinated animals. There has been much speculation that these MLVs have shed into the environment, exposing other animals, including wild animals, to these diseases (O’Driscoll, 2008).

Adjuvants and Preservatives: One other reason has been traced to the other substances that are in the vaccine vials that are potentially problematic. Additional components of the vaccines are the preservatives that do what preservatives do. These ingredients are also known in current medicine to be carcinogenic agents, including a compound called thymersol, a mercury derivative and aluminum, used to attenuate the viruses. The possible effects of aluminum are widely known. Even the cells these viruses are grown on can produce allergic reactions in the body. Some of the tissue lines used is from ducks, monkeys, pigs, and the like. These could be creating much of the constant itching, inflamed bowel, and eczematous ears that are so prevalent (O’Driscoll, 2008; McRearden, 2008; Blanco, 2008). There are additional ingredients called adjuvants. These are foreign proteins that are added to give a generic, non-specific immune response. These preservatives and adjuvants are what are believed to be the major cause of the surging incidence of fibrosarcomas in cats. Studies at Colorado State University by Professor Dr. Dennis Macy showed this strong correlation. It is felt by the biologics companies that if the body does not respond to the numerous viruses that are in each vial of vaccine, than surely the body will respond to other foreign proteins (O’Driscoll, 2008; McRearden, 2008; Blanco, 2008).

Introduction of modified live virus (MLV) vaccines and combination MLV vaccines: According to Dodds (1993), “Many veterinarians trace the present problems with allergic and immunologic diseases to the introduction of MLV vaccines some twenty years ago”. Dodds (1993) believed that MLV vaccines and combination MLV vaccines are the causes of the significant increase in autoimmune and allergic disorders in companion animals over the past 20 years or so. Also, according to Dodds (1993), “Combining viral antigens, especially those of MLV type which multiply in the host, provides a stronger antigenic challenge for the animal. This is often viewed as desirable because a more potent immunogen presumably mounts a more effective immune response. However, it can also overwhelm the immuno-compromised or even a healthy host that is continually bombarded with other environmental stimuli. This scenario may have a significant effect on the recently weaned young puppy or kitten that is placed in a new environment”.

Vaccination factors:
Process of injecting viruses (route of vaccination) and frequency of vaccinations: The process of injecting viruses into the body is a very unnatural method of introducing viruses, with the exception of Rabies virus. Most other forms of exposure are through the mucous membranes - the nose, throat/mouth, even the eyes. This creates another huge insult to the immune system. First
we gather a whole bunch of viruses and other 'stuff', and
then we inject them into the body at one time
(Glickman, 2000). According to Dodds (1993), "furthermore, while the frequency of vaccinations is
usually spaced two to three weeks apart, some
veterinarians have advocated vaccination once a week in
stressful situations. This practice makes no sense from a
scientific or medical perspective. While puppies exposed
this frequently to vaccine antigens may not demonstrate
over adverse effects, it is clear that their immune systems
may still be immature. Consequences later in life may be
an increased frequency of chronic debilitating diseases". However, further research is indicated, but these findings
should have raised concern.

**Hormonal status of the patient:** According to
Dodds (1993), “veterinarians and vaccine manufacturers
have paid relatively little attention to the hormonal status of
the patient at the time of vaccination. Because of the
known role of hormonal change along with infectious
agents in triggering autoimmune disease, vaccinating
animals at the beginning, during, or immediately after an
estrus cycle is unwise. Similarly, vaccinating an animal
during pregnancy or lactation can be fraught with
problems, not only for the dam but also because a
newborn litter is exposed to shed vaccine virus. One can
even question using MLV vaccines on adult animals in
the same household because of exposure of the mother
and her litter to shed virus.”

**Immune system overload:** When these viruses are
injected into the body, they find their way into the small
capillaries, then into the larger vessels and are filtered by
the lymph nodes. This sounds fine except that usually
these viruses are first introduced into the mouth and nose,
where the humoral immune system is stimulated. It
produces the powerful immunoglobulins (IgA, IgG, IgM),
which provide the first line of defense. When this primary
defense mechanism of the humoral immune system is
bypassed, the body becomes dependent on the cellular
immune system only; this is the branch that produces
antibodies. Producing antibodies is a fine thing, but when
the natural pathways are bypassed it creates an extra load
on the system. Having the natural stimulation of both
wings of the immune system is a more balanced approach
and is not what happens with injected vaccines
(O'Driscoll, 2008; McRearden, 2008; Blanco, 2008).

**Production of autoantibodies:** If autoantibodies are
being produced against laminin (coats kidney cells), then
one would not be surprised if kidney damage followed a
vaccine event. Similarly, if vaccines stimulate the
production of autoantibodies to DNA, then the vaccine
may well be introducing genetic defects. According to
O'Driscoll (2008), one representative from a major
biologies company, at a meeting on vaccines in 1997 said
quite embarrassed, “we know how to turn the immune
system on, but we do not know how to turn it off”.

**Nutritional factors:** For example, pantothenic acid
(vitamin B5) is vital for the production of anti-stress
hormones. According to O'Driscoll (2008), in a study
conducted on puppies starved of vitamin B5, and then
vaccinated, all died. B5 is a highly unstable vitamin,
easily destroyed by heating and freezing. Without B5 in
the diet, which is vital for the production of anti-stress
hormones, an organism is less able to survive the vaccine
challenge. Similarly, insufficient vitamin C and zinc in a
diet can render an organism unable to deal with stress.
Vaccines, of course, are designed to stress the body so
that it mounts an immune response and develops
antibodies against a pathogen (O'Driscoll, 2008).

**Environmental factors:** Last, but certainly not the least,
is the environmental factor. According to Dodds (1993),
"Some veterinarians trace environmental factors may have
a contributing role, the introduction of these vaccine
antigens and their environmental shedding may provide
the final insult that exceeds the immunological tolerance
threshold of the pet population”. Animals can react to
vaccines at any age. They might be vaccinated for many
years, without ill-effect, and then suffer an adverse
reaction. This has less to do with the animal's genes
(although it is well established that T cells can be
disrupted by vaccines), but to do with environmental
factors.

**Implications of these vaccine associated problems:**
According to O'Driscoll (2008), McRearden (2008) and
Blanco (2008), “Many veterinarians however, does not see
the need to report a suspected adverse reaction of
vaccines if an animal develops pancreatitis, arthritis,
ataxia, skin disease, epilepsy, behavioural problems,
alergies, colitis, etc., post-vaccination. At present time
there are no easy or effective reporting systems; many
vets are reluctant to report even those where an animal
dies, and the cause-effect relationship is not always clear.
Even to those who believe that many of the illnesses we
see, both acute and chronic, are directly related to over-
vaccination, it is still at times difficult to show how this
works”.

**Financial implication of this vaccine associated
problems:** The financial implication of this vaccine
associated problems could be alarming for the veterinary
profession, and even more alarming for the vaccine
industry. However, there are many options available for
the veterinary profession to consider. The first option is to
conduct blood tests to assess the presence of circulating
antibodies. According to O'Driscoll (2008), vaccine
companies have been using this measure as a marketing tool for decades. Now that they run the risk of losing booster income, however, they claimed that circulating antibodies are no measure of immunity. This is true, of course, but titre testing does show that the organism has been exposed to the virus without succumbing to it, and represents a safer alternative to over-vaccination. And so it seems that the best option would be for the profession to examine the frequency at which vaccines are administered, and become more aware of contraindications. From an income perspective, this would represent the best option for vets.

Vaccination boycott: In recent time, pet owners are becoming more informed about the vaccine issue, and many are choosing not to vaccinate at all. The other question concerns the vaccination of diseases that are not seen at all in a particular region, or that vaccines are not effective against because the viruses have many different serovaritants. Many have been ridiculed for refusing vaccination for themselves or their children, but considering the occurrences of short-term adverse effects and questionable efficacy, possible long-term health damage, and now also facing the potential of wide-ranging loss of civil liberties, is it so surprising that many are questioning what the actual benefits are surrounding most vaccination protocols? Are the cases of damaged children, non-functional adults, and the huge increases in cancer rates, immune and chronic diseases to be simply and blindly accepted by the public as tolerable losses? (O'Driscoll, 2008; McRearden, 2008; Blanco, 2008).

Where do we go from here? It seems obvious that there needs to be a new and open dialog regarding vaccines among the regulatory agencies, manufacturers, research and medical community, and the public. As a citizen with a right to good health, please be advised of the following issues. Vaccine quality in the U.S. (therefore in the world) relies for the most part, on manufacturers reporting to the FDA. Here is a relevant statement from the CDC: Manufacturers are required to submit the results of their own tests for potency, safety, and purity for each vaccine lot to the FDA. They are also required to submit samples of each vaccine lot to FDA for testing. However, if the sponsor describes an alternative procedure which provides continued assurance of safety, purity and potency, CBER may determine that routine submission of lot release protocols (showing results of applicable tests) and samples is not necessary (O’Driscoll, 2008; McRearden, 2008; Blanco, 2008). Yes, this is the scope of the quality-control protocol that oversees a market worth billions of dollars, yet allowing all these contaminates into the vaccines. It may be helpful to have an idea of the scope of the operation to understand what to deal with. We are advised that large-scale cell culture operations for biotechnology products use millions of litres of complex media and gases as well as huge quantities of organic and inorganic raw materials. These raw materials must always be assumed to contain contamination by adventitious agents. And because there are a potentially large number of animal and human viruses (or viral segments) that could be entering into the final vaccine products, it would take an equally large bank of molecular probes, as well as frequent, wide-spread testing, to screen for presence of these contaminating agents. This would obviously add time and expense for the manufacturers (O’Driscoll, 2008; McRearden, 2008; Blanco, 2008). What needs to be decided is this, is the effort and cost involved in cleaning up these admittedly filthy medical products, worth the resultant benefit to the public health? And since certain animal products are necessary for the production of vaccines, it may also be necessary to clean house at several levels, including the agricultural sector. It is no secret for instance, that commercial chicken flocks raised for meat and eggs are often carrying infectious avian leukosis virus, mentioned earlier (O’Driscoll, 2008; McRearden, 2008; Blanco, 2008).

Current initiatives and recommendations for control of vaccine-associated problems the Vaccine Adverse Event Reporting System (VAERS): VAERS is the principal passive surveillance system for vaccine adverse events in the United States. Vaccination may be coincidentally, not causally, related to the adverse event reported to VAERS. Accurate reporting to VAERS including review of medical records, and the use standard definitions for adverse events would improve causality assessments (Varricchio et al., 2004; Loughlin et al., 2009).

The Vaccine Safety Datalink: The Vaccine Safety Datalink is a collaborative effort of eight Medical Care Organizations (MCOs) and the Centers for Disease Control, which conducts post-licensure vaccine safety monitoring. Data from nearly nine million MCO members are evaluated weekly, comparing adverse events (AEs), which occur after vaccination to identical medical outcomes, which occur among appropriate controls (Lewis et al., 2009). Sequential (repeated) data analyses require a balance between the need for timely detection of real vaccine safety risks and the need to minimize false alarms (Lieu et al., 2007). Lewis et al. (2009) presented a new approach to sequential analysis, which uses exact statistics, which are especially helpful when there are small numbers of AEs, and/or variation over time in the ratio of exposed (vaccinated people) to unexposed (controls). This new method is simple, flexible, consistent with well-established statistical theory and suitable to population-based surveillance where, unlike in clinical trials, vaccine coverage varies in unpredictable ways.
across subgroups and over time (Lieu et al., 2007; Lewis et al., 2009).

CDC’s Immunization Safety Office (ISO) Scientific Agenda/ National Vaccine Advisory Committee (NVAC): In response to an Institute of Medicine recommendation (2005), CDC’s Immunization Safety Office (ISO) developed a draft 5-year Scientific Agenda (Agenda) and asked the National Vaccine Advisory Committee (NVAC) to review it (CDC, 2006). The goal of the Agenda is to identify and prioritize future ISO vaccine safety studies (Broder et al., 2009). During 2007-2008, ISO reached out to 45 scientists in academia, government, and industry at three meetings and conducted a selected literature review to identify vaccine safety science gaps that ISO could address. Criteria for including topics in an initial list were an ability to frame a vaccine safety hypothesis that was consistent with ISO’s mission, where a study could be initiated within five years. ISO grouped topics into specific questions or thematic areas and reviewed this second list with ISO-sponsored researchers and CDC scientists to define possible research needs (CDC, 2004; Broder et al., 2009). The initial list included 351 topics; 129 met inclusion criteria. The second list identified 18 specific questions and 42 thematic areas. Reviews yielded 30 possible research needs for the draft Agenda. Seven were specific vaccine safety questions (e.g., risk for neurological deterioration after vaccination in children with mitochondrial dysfunction and wheezing after live attenuated influenza vaccine). Twenty-three were thematic areas around vaccines/vaccination practices (e.g., simultaneous vaccination, nonantigen vaccine components), special populations (e.g., pregnant women, premature infants, immunodeficient persons), and clinical outcomes (e.g.,encephalitis/encephalopathy, neurodevelopmental disorders, postimmunization fever). Though a deliberative process, ISO defined 30 possible vaccine safety research needs. Further work will focus on defining specific questions in the thematic areas and prioritizing research topics. CDC will finalize this first comprehensive vaccine safety Agenda after receiving input from NVAC, stakeholders, and the public (Broder et al., 2009).

The Alliance for rabies control: The Alliance for Rabies Control strongly favors post-exposure immunization, as well as prophylactic vaccination, but points out that post-exposure immunization is not a rabies suppression strategy, because it does not neutralize the host reservoir (Clifton, 2007).

The World Health Organization (WHO) Global Immunization Vision and Strategy (GIVS): WHO-GIVS is a joint initiative with UNICEF. Its purpose is to identify opportunities that can be created to improve immunization service delivery and accelerate the availability of new vaccines or vaccines that were previously not available to the poorest countries of the world. According to Salisbury (2009), unlike previous immunization initiatives of WHO, GIVS includes vaccines for individuals of all ages, thereby breaking out of the previous constraints on vaccines for young children. GIVS has four main aims: immunize more people against more diseases, introduce a range of newly available vaccines and technologies, integrate other critical health interventions with immunization, and manage vaccination programs within the context of global interdependence (Salisbury, 2009). The period over which GIVS will operate spans 2006 to 2015.

Emergency prevention-system for transboundary animal and plant pests and diseases (EMPRES): Welte and Vargas-Terán (2004) briefly described Food and Agriculture Organization (FAO) EMPRES Livestock, its vision, its mission, and its activities to assist FAO developing member countries and regions in improving the ability of veterinary services to reduce the risks of introduction and/or dissemination of transboundary animal disease, by preventing, controlling, and eradicating those diseases, assisting countries in building their own surveillance/early warning systems, establishing contingency plans, and establishing a global information system for disease monitoring.

Pan-african programme for the control of epizootics (PACE): The potential partnership between the normative function of the Food and Agriculture Organization (FAO) in developing and promoting emergency preparedness and the implementation of improved national and regional disease surveillance by PACE and other partners could witness the commencement of more progressive control of epidemic diseases in Africa and greater self-reliance by African countries in coping with transboundary animal disease emergencies (Roeder et al., 1999).

American Animal Hospital Association (AAHA) recommendations: Vaccines are intended to be administered to healthy dogs - it is an advisory issued on vaccine labels, in veterinary literature and guidelines, as a dog's health status can have an impact on a vaccine's effectiveness and fail to elicit an immune response. Startlingly, the AAHA task force indicates that vaccination in a "severely immunosuppressed" dog can result in the dog acquiring the disease it is being vaccinated to prevent (ODRiscoll, 2008; McRearden, 2008; Blanco, 2008). According to AAHA's (2006) "As with pregnant dogs, veterinary medicine has advised against vaccination during illness, due to concerns about suboptimal sero-conversion, or worse, conversion of vaccine to disease." In other words, if you vaccinate a pregnant or sick dog, not only do you run the risk of a less-than-desirable immunological response, but you run the risk of your dog contracting the disease it is being
vaccinated against (O'Driscoll, 2008; McRearden, 2008; Blanco, 2008). Also, according to AHA (2003a), “...an attenuated pathogen in a host which is severely immunosuppressed, or genetically more susceptible, may result in the vaccine causing the disease for which it was designed to prevent." AHA (2003b) cautioned that: "When vaccinating an animal, the age of the animal, the animal's immune status, and interference by maternal antibodies in the development of immunity must be considered. Research has demonstrated that the presence of passively acquired maternal antibodies significantly interferes with the immune response to many canine vaccines, including CPV [parvo], CDV [distemper], CAV-2 [hepatitis] and rabies vaccines."

Advisory Committee on Immunization Practices (ACIP) recommendations: ACIP recommends that prophylaxis for the prevention of rabies in humans exposed to rabies virus should include prompt and thorough wound cleansing followed by passive rabies immunization with Human Rabies Immune Globulin (HRIG) and vaccination with a cell culture rabies vaccine (Manning et al., 2008). For persons who have never been vaccinated against rabies, postexposure antirabies vaccination should always include administration of both passive antibody (HRIG) and vaccine (Human Diploid Cell Vaccine (HDCV) or Purified Chick Embryo Cell Vaccine (PCECV)). Persons who have ever previously received complete vaccination regimens (pre-exposure or postexposure) with a cell culture vaccine or persons who have been vaccinated with other types of vaccines and have previously had a documented rabies virus neutralizing antibody titer should receive only 2 doses of vaccine: one on day 0 (as soon as the exposure is recognized and administration of vaccine can be arranged) and the second on day 3 (Manning et al., 2008). HRIG is administered only once (i.e., at the beginning of antirabies prophylaxis) to previously unvaccinated persons to provide immediate, passive, rabies virus neutralizing antibody coverage until the patient responds to HDCV or PCECV by actively producing antibodies (Manning et al., 2008). A regimen of 5 1-mL doses of HDCV or PCECV should be administered intramuscularly to previously unvaccinated persons. The first dose of the 5-dose course should be administered as soon as possible after exposure (day 0). Additional doses should then be administered on days 3, 7, 14, and 28 after the first vaccination (Manning et al., 2008). Rabies pre-exposure vaccination should include three 1.0-mL injections of HDCV or PCECV administered intramuscularly (one injection per day on days 0, 7, and 21 or 28). These recommendations involve no substantial changes to the recommended approach for rabies postexposure or pre-exposure prophylaxis. According to Manning et al. (2008), modifications were made to the language of the guidelines to clarify the recommendations and better specify the situations in which rabies post- and pre-exposure prophylaxis should be administered. No new rabies biologics were presented, and no changes were made to the vaccination schedules (Manning et al., 2008). However, rabies vaccine adsorbed (RVA, Bioport Corporation) is no longer available for rabies postexposure or pre-exposure prophylaxis, and intradermal pre-exposure prophylaxis is no longer recommended because it is not available in the United States (Manning et al., 2008).

Considering the alternatives: According to Blanco (2008), “The body has incredible capacity to provide protection against all sorts of invaders. So, if our approach to protection is from the standpoint of supporting the body in doing its job, which it already knows how to do, we are working at a more fundamental level. If we support the energy and physical systems of the body we will support the immune system, not overload it. Clean hygiene, good nutrition, clean water, plenty of exercise, constitutional treatments (preferably homeopathic), good breeding practices, and homeopathic nosodes, where needed (Blanco, 2008)”. Also, DNA vaccination remains a promising means to develop effective vaccines against infectious diseases and cancer. However, their poor performance in primates has demanded the use of genetic adjuvants or improved delivery techniques. One of our approaches to improving vaccine potency is to exploit genetic adjuvants. Although cytokine, chemokine and growth factor adjuvants have shown promise, it is believed that the most potent genetic adjuvants will be those that mimic the inflammation and Dendritic Cell (DC) maturation caused by natural infections (Bagley, 2009). Devising an appropriate technology for human rabies immunization includes new regimens of administration, one of which, a revised intramuscular regimen requiring only four doses and three clinic visits, proved highly efficient for postexposure treatment.

Current trends in rabies vaccines for global rabies control and possible eradication: In the 1920s, long before the recognition of bat and other wildlife rabies and the availability of modern vaccines, rabies in Japan was successfully controlled through mass vaccination of dogs (Wu et al., 2009). In the 1980s, large-scale oral vaccination campaigns were first used to fight the rabies epidemic successfully in foxes (Eisinger and Thulke, 2008). Nowadays, fixed-wing aircraft deliver vaccine-filled bait pieces, recording every drop with GPS precision (Eisinger and Thulke, 2008). However, there is still a debate on how many baits per unit area should be administered (WHO, 2005), what percentage of the fox population needs to be immunized to ensure control success and whether there is a threshold involved (Lloyd-
In 1981, a population model predicted the threshold level to be about 70% for central European densities of about 3 foxes per km² (Eisinger and Thulke, 2008). Subsequently, large-scale and long-term oral vaccination programmes in Europe were prepared to implement this target level following WHO/OIE guidelines.

Morbidity and Mortality Weekly Recommendations and Reports (MMWR) in 1993 consolidates the deliberations of the International Task Force for Disease Eradication (ITFDE), which was convened six times from 1989 through 1992 to evaluate diseases as potential candidates for global eradication (CDC, 1992a, b; WHO, 1993). CDC supports the findings, which indicated a need for greater recognition of the potential to eradicate targeted diseases. Three reports, covering results of the first five meetings of ITFDE, were published previously in the MMWR (CDC, 1992a, b), and reprinted in WHO's Weekly Epidemiological Record (WHO, 1993). Large-scale eradication of rabies using recombinant vaccinia-rabies vaccine was reported (Wu et al., 2009). An immunized dog population can be a solid barrier to prevent rabies from spreading to humans (Cleaveland et al., 2006; Zinsstag et al., 2007; Wu et al., 2009). Rabies pre-exposure vaccination and post-exposure treatment is recommended for occupationally exposed persons. Some European countries have already adopted recommendations through specific protocols. Treatment of international travellers after bat bites is also recommended. The promoting of research programmes on bat rabies in Europe is underway (Stantic-Pavlinic, 2005).

According to Clifton (2007), Dr Oscar Pedro Larghi (Argentinian Medical Director) reported to the members of the International Society for Infectious Diseases in May 1998, that “control of rabies in developing countries can be very successful if based on appropriate planning, health education of human populations, 70% vaccine coverage of dog populations, and epidemiological surveillance (Clifton, 2007). However, recent outbreaks of rabies highlight the fact that rabies is a transboundary disease and can reemerge in areas where successful control programs have been active for many years (Cohen et al., 2007). Summarily, ORV has evolved as a significant adjunct to conventional rabies prevention and control efforts and has resulted in the successful elimination of canine rabies from coyotes in the U.S.A review of the progress in domestic animal control in the U.S. over the past 50 years was made by Ben Sun of the California Department of Health. Building upon such achievements, efforts are underway to repeat this success in developing countries throughout the world (Rupprecht and Tumpey, 2007).

Trends and efforts in control of rabies across the continents: According to Sobota (2008), “The creation of the One Health Challenge in support of World Rabies Day reflects the new generation of health professionals’ desire to act at the local level in order to affect our global health. The One Health definition, established by the paraprofessional One Health task force, recognizes the confluence of environmental, animal, and human interactions. The monumental amount of energy amongst the paraprofessional health team recognizes that there is no more important disease to begin educating and developing our efforts than rabies. World Rabies Day is a testimonial to the One Health concept. Our cooperative efforts are infiltrating the larger communities throughout the world and continuing to reduce cases of preventable rabies. World Rabies Day provides an opportunity for the human spirit to significantly impact the lives of others in a positive way”.

America: Progressive elimination of rabies in wildlife has been a general strategy in Canada and the United States; according to Sterner et al. (2009) common campaign tactics are Trap-Vaccinate-Release (TVR), Point Infection Control (PIC), and ORV. These tactics have proven crucial to elimination of raccoon rabies in Canada and to maintenance of ORV zones for preventing the spread of raccoon rabies in the U.S. (Sterner et al., 2009). In recent years, cases of rabies among humans in urban areas (transmitted by domestic animals) have declined considerably in the Americas (Salmon-Mulanovich et al., 2009). This is likely the result of an aggressive initiative by the member states of the Pan American Health Organization (PAHO) to eliminate urban rabies in the Americas (Navarro et al., 2007; Salmon-Mulanovich et al., 2009). According to Miguel Escobar, M.D., associate director of Merial Inc., (which is the world’s largest manufacturer of anti-rabies vaccines), “In 1990 there were 16,464 reported cases of canine rabies in Latin America. In 1998 that was reduced to 2,608. Human rabies cases were reduced from 252 to 74.” Most of the rabies case reduction was in Buenos Aires, Lima, and Sao Paolo, all of which completely eliminated rabies by vaccinating from 60 to 80% of their estimated dog populations during a series of three-month campaigns directed by Larghi in Argentina (Clifton, 2007).

In Canada, before rabies control programs were implemented, red foxes accounted for ≈45% of all rabies cases in Ontario (Rosatte et al., 2007a). During 1989-1995, ORV was used in Ontario to progressively eliminate arctic fox-variant rabies that had spilled into (i.e., had been transmitted to another species) red foxes and spread southward (Sterner et al., 2009). The last report of a rabid fox in metropolitan Toronto was in 1996 (reporting period through September 2006), which confirms that distributing ORV bait is a feasible tactic for the control of rabies in foxes in urban environments (Rosatte et al., 2007a). The ground and aerial distribution
of rabies vaccine bait in metropolitan and greater metropolitan Toronto, which resulted in immunization of a substantial portion of the fox population against rabies, eliminated raccoons from that urban complex (Rosatte et al., 2007a). Greater metropolitan Toronto has been free of reported cases of rabies in red foxes for a decade (1997-2006) and is a notable success for the Ontario Ministry of Natural Resources rabies control programs (Rosatte et al., 2007a). To eliminate raccoon-variant raccoons from the Ontario, a Point Infection Control (PIC) tactic, which integrated population reduction (PR; sometimes referred to as culling or depopulation), TVR, and ORV, was implemented (and eliminated the variant from Ontario (Sterner et al., 2009). Elimination of raccoon rabies from Wolfe Island at the mouth of the St. Lawrence River using similar tactics was recently reported (Rosatte et al., 2007b). Additionally, to prevent raccoon rabies from reemerging in southern Ontario, ORV baiting for raccoon-variant raccoons continues in northern New York (Sterner et al., 2009).

Several brands of rabies vaccine are available in the United States (CDC, 2006). Oral rabies vaccination programs have been implemented to control the spread of wildlife rabies in the United States. However, current surveillance systems are inadequate for the efficient management and evaluation of these large scale vaccine baiting programs (Blanton et al., 2006). U.S. CDC rabies program Chief Charles Rupprecht on World Rabies Day formally pronounced the U.S. free of canine rabies, but similar informal proclamations have been issued for years (Clifton, 2007). Rabies in small animals has been dramatically reduced in the U.S. since the introduction of rabies vaccine baiting of domestic animals in the 1940s. As a consequence, the number of human rabies cases has declined to only a couple per year. During the past several years, the dog rabies variant has almost disappeared completely (Lackay et al., 2008). Progress has also been made in containing gray fox raccoons in Texas (Rupprecht and Tumpey, 2007). An attempt begun a year earlier to eradicate coyote raccoons in Texas, by airdropping vaccine bait pellets, achieved a 98% reduction of canine rabies in all species by 1998 (Clifton, 2007). Also, in Texas, the use of ORV stopped the northward spread and led to the progressive elimination of the DDC variant of raccoons in coyotes (Canis latrans). Progress has also been made in preventing the spread of raccoon rabies from the eastern U.S. Nevertheless, the most profound challenge facing the program is the need for baits and oral vaccines with improved effectiveness in other mesocarnivore reservoir species, such as skunks and mongoose (Rupprecht and Tumpey, 2007). The preventive vaccination approach also works in wildlife. Anne Arundel County, Maryland, for example, had 97 cases of animal rabies in 1997, when county officials began experimentally distributing oral rabies vaccine pellets to immunize raccoons. Gradually expanding the program, the county had just 10 animal rabies cases in 2006 (Clifton, 2007).

In Mexico, the national rabies control programme using mass parenteral vaccination of dogs started in 1990. As a result of the mass dog vaccination campaigns in Mexico, human rabies cases due to dog-mediated rabies decreased from 60 in 1990 to 0 in 2000. The number of rabies cases in dogs decreased from 3,049 in 1990 to 70 cases in 2007 (Lucas et al., 2008). On the basis of rates of spread of 30-60 km/year in the Mid-Atlantic states before 1997 (Slate et al., 2005; Krebs et al., 2005), ORV is viewed as having slowed movement of the virus and, with contingency actions to eliminate some dispersed cases, prevented westward spread of raccoons among raccoons (Sterner et al., 2009). Still, enhanced and public health surveillance indicate that areas in the North America west of the Appalachian Ridge remain free of raccoon-variant raccoons (Slate et al., 2005; Krebs et al., 2005; Blanton et al., 2008; Sterner et al., 2009). Canada, Mexico, and the United States continue to work toward a North American Rabies Management Plan that will enhance rabies surveillance and control on a continental framework (Rupprecht and Tumpey, 2007).

Europe: There have been many changes and accomplishments in rabies control and prevention since the first International conference "Rabies in Europe" was held in Kiev on June 15-18, 2005 (Briggs, 2008). The elimination of raccoons was demonstrated following oral vaccination of foxes in Western Europe, where red foxes are the reservoir host (Clifton, 2007). However, empirical evidence indicates that, in regions with less then 70% immunization coverage, raccoons still have been eliminated (Bugnon et al. 2004). The immediate challenge for rabies control is to stockpile enough vaccines for mass dog vaccination campaigns (Wu et al., 2009). According to Harris et al. (2006), in a study on passive surveillance for EBLVs in the UK and no active infection with EBLV type 1 was recorded in their (Harris et al., 2006) study. In Europe, primary efforts should be focussed on the implementation of effective passive and active surveillance systems for EBLVs in bats (van der Poel et al., 2006). Two countries in the Central and Eastern Europe are currently rabies free, and several others are close to being rabies-free (Matouch, 2008). Due to improvement and adaptation of vaccination strategies that took into consideration the peculiar topographical features of a fragmented landscape and the high fox densities, the number of rabies cases in North Rhine Westphalia, Germany decreased in 2001 (Müller et al., 2005). The breakthrough in rabies control in the Saxony region of the Czech Republic came when continuous annual trilateral meetings with the countries involved were initiated which led to a considerable improvement of the vaccination strategies in the adjacent areas to Saxony. According to Müller et al. (2005), for more than three and a half years no rabies case has been reported from this region.
In 1993, the decision was made to apply ORV of the red fox as a method of rabies control in Poland. ORV was undertaken twice per year (spring and autumn). In 2002, the vaccine campaign covered the whole territory of Poland. According to Smreczak et al. (2008), after 13 years of ORV, rabies incidence decreased sharply, from 3,084 cases in 1992 to 82 in 2006 (a 97% decrease in the number of rabies cases). The epidemiological situation in 2006 points to the constant decrease of rabies cases as a result of ORV. The progress made over the past decade demonstrates that Poland is meeting the requirements to eliminate rabies in terrestrial animals (Smreczak et al., 2008). The last endemic case of rabies in Switzerland was diagnosed in 1996 after an adaptation of the vaccination strategy (Zanon et al., 2000). The last instance of rabies in a native French animal was reported in 1998 (Peigue-Lafeuille et al., 2004). According to Peigue-Lafeuille et al. (2004), vaccination is performed in official rabies centers in France. In Germany, ORV of foxes using MLV vaccines offered a new method of rabies control in wildlife (Müller et al., 2005). With the enlargement of vaccination areas in West Germany reaching a maximum size of about 215,000 km² in 1995, the policy of using ORV became increasingly successful and rabies incidence decreased drastically in subsequent years (Müller et al., 2005). As a consequence, in the eastern parts of Germany, a rapid decrease in the number of rabies cases was observed in the early 1990s after the implementation of ORV. These eastern regions have been free of rabies for more than 10 years (Müller et al., 2005). As a result of ORV, the rabies incidence drastically decreased during the past 20 years from 10,484 rabies cases in 1983 to 56 in 1999; the lowest number of rabies cases ever reported in Germany.

Summarily, once large-scale vaccination was applied in the western regions, rabies was quickly eliminated. During the past 10 years, as in other European countries, the efficacy of oral fox vaccination campaigns has been increased by a permanent adaptation and optimization of the vaccination strategy based on analysis of the prevailing conditions and recent scientific perceptions. These measures have included (i) den baiting, (ii) double baiting (repeated aerial distribution of baits 14 days after the first vaccination campaign in the same area using perpendicular flight lines with a distance of 1000 metres), (iii) summer vaccination, (iv) an increase of bait density and (v) a reduction of flight lines (Müller et al., 2005).

Asia: Rabies control has not be effective in Central Asia (Gruzdev, 2008). The only country with a steady decline in Asian countries is Thailand, where the number of cases has decreased from around 200 to about 20 cases per year. The most dramatic changes were observed in China. Human rabies cases declined from around 5,000 cases per year in the 1980s to about 160 in the mid-1990s. During the past 20 years in this county, 15,000 persons received rabies postexposure prophylaxis after dog bites, but no rabies occurred. According to Zhenyu et al. (2007), no human rabies cases followed exposure to dogs that were within 50 km during 2002-2004. However, these trends have since been reversed. A steady increase has been reported over the past 10 years with more than 3,200 cases reported in 2006. Although there are many factors that contribute to the epidemic or endemic nature of rabies in these countries, the single most important factor is the failure to immunize domestic dogs, which transmit rabies to humans (Fu, 2008). These reemerging rabies in China has led to a carrier-dog myth and strict pet population control policies (Wu et al., 2009).

Recently, according to Nadin-Davis et al. (2007), a national rabies survey in India, based on clinical diagnosis and sponsored by the WHO, found that 20,000 persons died of rabies each year (Sudarshan et al., 2006). These observations indicate a great need to strengthen laboratory diagnostic capabilities for rabies in India and to use genetic typing to improve knowledge of the nature of the viruses that circulate in India. Using several positive samples identified by this method, Nadin-Davis et al. (2007) studied the epidemiologic origins of rabies from multiple areas of the country. The resulting increase in disease surveillance would help justify subsequent control measures. Accordingly, molecular methods for rabies virus detection have been introduced to the National Institute for Mental Health and Neurosciences in Bangalore, India (Nadin-Davis et al., 2007). In Japan, no rabies case has been reported for about 50 years. Since 1957 Japan has successfully eradicated human and animal rabies through registration, confinement and compulsory vaccination of family dogs, and elimination of stray dogs. This was successful since the government started the pre-exposure vaccination (Tamura et al., 2007). Pre-exposure prophylaxis recommended by WHO consists of 3 doses given intramuscularly on days 0, 7, and 28, making it possible to complete pre-exposure prophylaxis in one month (Yanagisawa et al., 2008). Summarily, most countries in Asia like China is planning increased investment in rabies surveillance and prevention that will include recommended laboratory support and should help alleviate this situation in the future (Childs et al., 2007; Zhenyu et al., 2007).

Africa: Although industrialized countries have been able to contain recent outbreaks of zoonotic diseases through enhanced mass vaccination initiatives, many resource-limited and transitioning countries have not been able to react adequately (Zinsstag et al., 2007). Cross-sectoral assessments of interventions such as mass vaccination of dogs for rabies in Chad consider human and animal health sectors from a societal economic perspective (Zinsstag et al., 2007). Unfortunately, dog vaccination is difficult in many developing countries like Mongolia and
Chad because of high dog turnover rates, shortages of funding and personnel, and competing priorities (Cohen et al., 2007). Also, results from research projects in eastern Africa show that mass vaccination of domestic dogs has the same result, even in areas such as the Serengeti ecosystem, which comprise a wide diversity of wildlife species. When sufficient domestic dogs are vaccinated, rabies also declines in wildlife, and human exposures to the rabies virus are significantly reduced (Clifton, 2007).

In South Africa where rabies had been well controlled for >10 years, Cohen et al. (2007) described an outbreak of human rabies in a province of South Africa. According to Cohen et al. (2007), in South Africa, central-point dog vaccination campaigns in villages in the affected area were intensified after identification of the increased numbers of rabies cases in domestic dogs. A community awareness program related to the hazards of dog bites and the importance of timely visits to the clinic for rabies postexposure prophylaxis was established in February 2006. Furthermore, healthcare workers were educated regarding appropriate management of dog bites. Vaccine and immunoglobulin availability was improved by increasing the number of facilities providing the vaccine and by ensuring that patients did not have to pay for treatment (Cohen et al., 2007). The combined number of doses of human rabies vaccine used in Limpopo Province, South Africa in the public sector increased from 3,000 in 2004 to 6,000 in 2005 and 56,000 in 2006. Also, use of anti-RIG also increased over the same period with 100 doses given in 2004, increasing to 500 in 2005 and 2,500 in 2006 (Cohen et al., 2007). As a result, the number of human rabies cases in Limpopo Province, South Africa decreased after May 2006; no further human cases had occurred as of June 30, 2007. This decrease is likely due to the introduction of coordinated control measures (including aggressive PEP). An increased awareness of rabies after interventions for control may have contributed to increased case reporting after February 2006; this situation may have affected apparent trends in human case numbers and contributed to the delay in observed decline in dog cases (Cohen et al., 2007).

Seghiaiet et al. (1999) reported that puppies in Tunisia responded to vaccination with no significant interference by passive maternal immunity. Based on these percentages, a 93% rate of protection may be expected for vaccinated dogs. Their study confirms that all dogs (even those less than 3 months of age) must be vaccinated during mass campaigns. The expected protection conferred by locally produced potent vaccines reaches 79-99% based on the age of the dogs. In Nigeria where dog bites continue to be the main mode of transmission of the disease to man and remains a serious public health hazard, the most logical and cost-effective approach to rabies control is elimination of stray and owner less dogs combined with a programme of single mass immunization in the shortest possible time, at least 80% of the entire dog population (Awoyomi et al., 2007). Summarily, the key for controlling zoonoses such as rabies, echinococcosis, and brucellosis is to focus on the animal reservoir. In this respect, ministries of health question whether the public health sector really benefits from interventions for livestock (Zinssstag et al., 2007). Dog rabies control relies principally on the mass immunization of dogs in order to achieve population immunity levels sufficient to inhibit rabies transmission. In Africa, such high levels of population immunity are rarely achieved due to a number of reasons. Oral immunization has been shown to be an effective means of inducing high levels of immunity in fox populations in several European countries, and this technique has been mooted as a means of overcoming the logistical problems of delivering injectable rabies vaccines to dogs (Cohen et al., 2007). Although highly effective if administered correctly, PEP is much more costly than vaccination of domestic dogs (Rupprecht and Gibbons, 2004; Cohen et al., 2007).

Future trends in rabies vaccines and its roles and implication for global rabies control and possible eradication: Experts have recognized for decades that rabies is wholly eradicable from all species except bats through targeted mass immunization - and the chief obstacle to eradicating bat rabies is that no one has developed an aerosolized vaccine that could be sprayed into otherwise inaccessible caves and tree trunks. Inventing such a vaccine is considered difficult but possible (Clifton, 2007). Recently, the heroic recovery of an unvaccinated teenager from clinical rabies offers hope of future specific therapy (Rupprecht et al., 2006). While post-exposure vaccination is essential, and should continue, with improvement to achieve consistently positive results, progress toward eliminating rabies has been markedly faster in nations that have emphasized preventively vaccinating dogs (Clifton, 2007). In most countries, canine rabies was already close to elimination, but not because there were fewer dogs. Rather, canine rabies had nearly disappeared because unvaccinated street dogs had been replaced by an almost equal number of vaccinated pets (Clifton, 2007). As international concerns increased, several corrective actions have been implemented in many countries since 2005, which aimed at improving vaccination protocols and a consistent vaccination strategy in the respective federal states aiming to eliminate the residual focus (Müller et al., 2005). Many disease agents like rabies have developed strategies to overcome extremes of reservoir qualities like population size and density. Every infectious agent spreads easier when its hosts are closer together (Murphy, 2008). Recent epidemics of these rabies diseases have served as a reminder of the existence of infectious diseases and of the capacity of these diseases to occur unexpectedly in new locations and animal species.
In terms of the control of rabies, advances will come from the enhanced ability to control the spread of RABV among wild animal populations as well as the development of newer, more effective vaccine strategies (Rupprecht et al., 2004). Several new recombinant RABV vaccines are under development for potential use in animal populations. In addition, advances in the reverse genetics of these viruses should see the application of genetically engineered versions for a variety of therapeutic purposes. The recent report of a rabies survivor after drug-induced coma and antiviral treatment demonstrates the type of future milestones in need of basic and applied re-evaluation (Willoughby, 2005). As a result of the efforts in developing recombinant RABV as vaccine vectors and as cytolytic agents, it is likely that clinical trials of genetically engineered RABV in humans will take place in the near future. A number of issues need to be considered in the use of such agents, such as their safety for use in humans, as well as the protection of animal populations that may be exposed to such viruses. Nonetheless, the advances in understanding virus replication and pathogenesis should make it feasible to address these issues, so that these viruses that have long been a burden to humanity can instead be a benefit (Lyles and Rupprecht, 2007).

The potential for manipulation of the RABV genome has been simplified by the generation of infectious virus entirely from cloned cDNA. If future recombinant, replication-incompetent, inactivated, or DNA-based vaccines prove both efficacious and economical, they may render most previous biosafety concerns obsolete, paving the way for more widespread, free-ranging wildlife and dog rabies control, particularly in developing countries (Lyles and Rupprecht, 2007). At present, no evidence suggests that prophylaxis failure is caused by antigenic variation of RABV (WHO, 1992). Rather, vaccine failures are usually associated with inadequate wound care, omission of potent serum, failure to infiltrate the wound with immune globulin, delay, or failure to follow recommended procedures (Lyles and Rupprecht, 2007). Future tactics for global human rabies prevention will continue to focus on the need for enhanced public health communications, continuing professional education; potent, inexpensive pre- and post-exposure vaccines and new schedules; and viable alternatives to rabies immune globulin (e.g., monoclonal antibodies (Marissen et al., 2005)). Based on the recognition that rabies at its source can be effectively controlled and sometimes eliminated, safer, more effective, and inexpensive medical and veterinary vaccines are a necessity for animal reservoirs, vectors, or victims of the disease (Lyles and Rupprecht, 2007).

CONCLUSION

According to BBC News headlined worldwide on September 8, 2007, “Rabies could be gone in a decade”. “Rabies could be wiped out across the world,” “if sufficient vaccinations are carried out on domestic dogs, according to experts (BBC News, 2007; Clifton, 2007).” Though, rabies is indeed a typical zoonotic disease which has been known since ancient times; more than 4300 years (Takayama, 2008) and effective methods to treat rabies patients have not yet been available, the only means to escape rabies death is to receive the post-exposure prophylaxis of rabies with rabies vaccine as soon after animal bite as possible (Takayama, 2005). We should keep in mind that rabies is preventable but incurable and that vaccination is still the key to prevent rabies in small animals and rabies transmission to human beings (Lackay et al., 2008). Appropriate use of a highly effective vaccine can help eradicate a major disease when humans are the only natural host for the virus, and there is no natural reservoir or intermediate host. Live virus vaccines have played and continue to play a central role in these current eradication efforts (Graham and Crowe, 2007). Surveillance strategies for rabies viruses and other rabies-related viruses in Africa must be improved to better understand the epidemiology of this virus, roles of vaccines and its implication for global public health, and to make informed decisions on future vaccine strategies because current evidences are insufficient that current rabies vaccines provide protection against these other rabies-related viruses.

REFERENCES

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