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LA VACCINAZIONE NEL GATTO: PRO E CONTRO

FELINE VACCINATION: PROS AND CONS

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I n d e x

ACRONYMS

DOI: Duration Of Immunity

EBVM: Evidence-based Veterinary Medicine

FCV: Feline Calicivirus

FeLV: Feline Leukemia Virus

FIV: Feline immunodeficiency virus

FIP: feline infectious peritonitis

FHV-1: Feline Herpesvirus type 1

FISS: Feline Injection-Site Sarcoma

FPV: Feline Parvovirus

MDA: Maternally-derived antibodies

MLV: Modified Live Vaccines

VGG: Vaccination Guidelines Group

WSAVA: World Small Animal Veterinary Association

ABSTRACT

Vaccination is the most efficient and cost-effective method of controlling infectious diseases in humans and animals. Smallpox is an example of the effectiveness of using vaccines to eradicate a disease. Even today, many infectious diseases are kept under control by routine vaccination. In cats, parvovirus, calicivirus, herpesvirus type 1 and rabies are contained through vaccination and guidelines that provide veterinarians with protocols to safeguard public and animal health. The technology behind vaccine production in continuous improvement, a greater understanding of immune mechanisms and ways to optimize the immune response to achieve maximum protection have allowed more excellent safety and efficacy in the use of vaccines. These improvements allow for a reassessment of the risks and benefits of vaccination and have led to changes in guideline protocols. Vaccination, however, is not always a harmless, risk-free procedure. A risk-benefit analysis should accompany the use of any vaccine. An evaluation of the pros and cons carried out by the veterinarian in consultation with the animal owner allows the creation of personalized vaccination protocols based on the actual risk of exposure to an infectious disease, the animal's lifestyle, and age. Before using a vaccine, the veterinarian should consider the likelihood of an adverse effect and possible consequences. Adverse effects should be reported to the vaccine manufacturer or the appropriate state agencies. Although adverse effects are rare and, in most cases, mild and transient, every veterinarian should take care not to vaccinate more than necessary, following the guidelines and considering the possibility of an adverse effect each time.

INTRODUCTION

Vaccination is the most efficient and cost-effective method of controlling infectious diseases in humans and animals. Effective vaccination has allowed the eradication of diseases such as smallpox, cholera, and rinderpest. Still, it allows practicing the control and containment of diseases such as foot-and-mouth disease, distemper in dogs, feline and canine parvovirus, rabies and influenza. All this thanks to a constantly improving technology, modern molecular techniques, and a greater understanding of immune mechanisms and ways to maximize immune response and protection. (Tizard, 2013)

The term vaccine was coined for the first time by Edward Jenner[1] at the end of the 18th century to describe the inoculation of the cowpox virus (*Variolae vaccinae,* hence the term's origin) in a child to induce protection against smallpox. He observed that milkers who came into contact with the pus of cowpox, an infectious disease that mainly affected the udders of cows leading to the formation of pustules similar to those of smallpox, were immune to the virus that affected humans. Therefore, he took purulent material from the wounds of a woman suffering from cowpox and inoculated a child who later became immune to smallpox. This allowed the practice of "vaccination" to become a routine part of medicine and brought to light the close correlation between infectious animal diseases and human ones; today, we know that the smallpox and the cowpox both belong to the family of "*Poxviridae*". Problems with purity, safety and availability were common. Nevertheless, both the efficacy and the imperfections

[1] McVey S, Shi J. Vaccines in veterinary medicine: a brief review of history and technology. Vet Clin North Am Small Anim Pract. 2010 May; p.381-392

of the vaccination led to the final global eradication of smallpox and was the inspiration for developing products and programs of immunization against different diseases in humans and animals.

Luis Pasteur represented another important chapter in the history of vaccination. During his studies on fowl cholera, Pasteur observed how chickens first infected accidentally and then experimentally through cell cultures became resistant to the disease. Pasteur understood that if an organism in contact with a pathogen survives the disease, it develops a resistance to it, which today we call immunity, and this resistance becomes permanent. The need to make inoculated pathogens less virulent led to the creation of the concept of "attenuation", which can be achieved by serial passages of the pathogen in different animals or cell cultures or by processing it with heat or oxygen to make it harmless but at the same time able to elicit an immune response. The term vaccination was used for the first time in 1881 by Pasteur to indicate the use of immunogens against diseases other than cowpox. He studied the microorganism today known as *Bacillus anthracis* responsible for anthrax in ruminants and practiced vaccination on cattle with positive results. However, the most significant discovery of Luis Pasteur was the rabies vaccination in humans; specifically, he used the very first vaccine against rabies in a child bitten by an infected dog in 1885.

The work of Salmon and Smith (1886) demonstrated that some microorganisms could be wholly inactivated, then killed. These developments eventually led to successful immunization programs against such important diseases as typhoid fever, tuberculosis, and rinderpest. Gaston Ramon (1924), a biologist and veterinarian researcher at the Pasteur Institute (a French non-profit foundation

dedicated to the study of biology, microorganisms, diseases, and vaccines, of which Luis Pasteur was the first director), applied the principles of attenuation and inactivation extending them to tetanic toxins creating what today is called "toxoid", a toxin inactivated by heat and formalin. In addition, efficacy was improved by immersing the toxoid in an aluminium hydroxide, providing an adjuvant effect. These principles are still the fundamental pillars of modern vaccination, which has, however, achieved significant improvements with new generations of vaccines.

In the beginning, the vaccines were produced in regional research institutes, only later, between the '30s and '40s of the '900 began to produce vaccines on a large scale and then the industrialization of production processes. As a result, government authorities created regulatory frameworks that established regulations and guidelines for the registration of new biological products and regulation of the production of increasingly pure, safe, and potent vaccines. Based on these regulations, all batches of released vaccines are tested to ensure consistent formulation characteristics and immunological potency, safety, and purity (sterility and absence of contamination with foreign biological agents). The development of good manufacturing practice guidelines has further ensured the constant production of vaccines that will provide constant immunogenicity and efficacy. Therefore, any approved vaccine can be used with confidence by the veterinary clinician achieving the outcome expected and indicated on the vaccine by the manufacturer.

Before vaccination became a routine practice in veterinary medicine, processed immunoglobulins (usually by horse serum) were used. Immunoglobulins confer a type of immunity called passive immunity,

still used for post-exposure prophylaxis of rabies and tetanus but surpassed in terms of advantages by active immunity (conferred by vaccination) for immunological memory and reduced risk of infection. To date, the use of passive immunity has been significantly reduced.

During the mid-1950s, veterinarians used rabies vaccine as routine prophylaxis in dogs; subsequently, as medical technology advanced and thus development and production capacity increased, pet vaccination expanded to include rabies for cats, feline herpesvirus (FHV-1), parvovirus in cats (FPV) and dogs (CPV), and feline calicivirus (FCV). These vaccines include traditional inactivated antigenic formulations, multiple attenuated agents, and modern technologies such as recombinant vector vaccines with smallpox (using Canarypox virus for feline leukemia vaccine), subunit vaccines, and polynucleotide vaccines.

The World Small Animal Veterinary Association (WSAVA) has published guidelines: "GUIDELINES FOR THE VACCINATION OF DOGS AND CATS" compiled by the Vaccination Guidelines Group (VGG). The Vaccination Guidelines Group (VGG) is a study group for vaccination guidelines of the WSAVA that met to compile guidelines for the vaccination of both dogs and cats that are applicable worldwide. They were first published in 2007; an updated version came out in 2010.

To support the credibility and scientific evidence of the recommendations, the VGG has used the Evidence-based Veterinary Medicine (EBVM), which means that each recommendation is followed by an acronym that leads to a classification that defines the weight of scientific evidence so that every veterinarian is made aware of the nature of scientific evidence in support of the recommendation made.

The VGG has used its own classification, considering it more appropriate than the classic one, which is considered poorly applicable to the field of vaccinology. He classified the EBVM into four categories which are now quoted verbatim:

“Category 1 evidence: a recommendation supported by peer-reviewed scientific publication of either experimental or field data. Evidence within this category might still be of variable scientific quality despite peer review, as the peer review process does not conform to a universal standard.

Category 2 evidence: a recommendation supported by unpublished commercially sensitive studies submitted as part of a regulatory package for licensed veterinary vaccines. The assumption for this level of evidence is that information appearing on the datasheets of licensed products has been through competent peer review by regulatory authorities.

Category 3 evidence: a recommendation supported by commercial or independent experimental or field data that have not been published in the peer reviewed scientific literature or were not included in a formal regulatory package and subjected to scrutiny by regulators.

Category 4 evidence: a recommendation unsupported by experimental or field data but assumed from knowledge of the ‘first principles’ of microbiology and immunology or supported by widely-held expert opinion.

Throughout this document, statements may be followed by a qualifier (EB1), (EB2), (EB3) or (EB4) reflecting an ‘evidence base’ of category 1, 2, 3 or 4, respectively. For each occasion of use only the most

rigorous level of evidence available will be given.” (Day, Horzinek, & Schultz, 2015).

The VGG recognizes the significant differences in practice and relative costs in different parts of the world and therefore considers that the recommendations in the guidelines may be inapplicable in developing countries. Therefore, the VGG specifies that these recommendations are not obligatory but rather an indication for national associations and individual veterinary practitioners to prepare vaccination programs that also consider the local situation. However, the VGG recommends that all dogs and cats’ benefit from vaccination when the local situation makes it possible (Day, Horzinek, & Schultz, 2015). This concept is called "herd immunity". That is the resistance of a group of animals (or people) to a disease due to a percentage of animals (or people) immune present in that group. Herd immunity reduces the probability of an encounter between a susceptible subject and an infected one; this makes it possible to reduce and then slow down the spread of the disease for which it is vaccinated (Tizard, 2013).

Thus, considering the socioeconomic differences present worldwide, the VGG has defined "core" vaccines as those that all dogs and cats should receive, regardless of geographic location and circumstances relative to them. Therefore, they are "highly recommended" because they offer protection against serious, life-threatening diseases that have a worldwide distribution. Therefore, in the following chapters, it is reported which are the core vaccines for the cat (p.41-42). The modalities and times of administration recommended by the VGG consider one of the most common causes of ineffectiveness of a vaccine: the interference given by Maternally Derived Antibodies

(MDA). This topic will also be dealt with specifically in the following chapters (p.44).

The VGG supports the use of outpatient serological tests that determine the seroconversion to the components of "core" vaccines. However, these tests apply mainly to the core vaccines of the dog. Regarding the cat in the literature is reported a correlation between serum antibodies and protection of the subject only in the case of infection with Feline Parvovirus (FPV) (Day, Horzinek, & Schultz, 2015). Therefore, it is a practice that may be useful primarily for managing infectious disease outbreaks in catteries. The VGG recommends that vaccines not be administered where there is no demonstrated need. Scientific evidence has shown that an interval of 3 years between one vaccination cycle and the next is suitable to confer protection to the animal since the Duration Of Immunity (DOI) is many years and can last for the animal's entire life (Day, Horzinek, & Schultz, 2015).

The VGG has defined "non-core" vaccines as required based on how much the animal's geographic location, local environment, and lifestyle increase the risk of contracting certain infectious diseases. Lastly, the VGG has classified certain vaccines as "non-recommended" when scientific evidence is insufficient to justify their use. The VGG supports the concept of annual follow-up visits that emphasize annual re-vaccination and, consequently, the client's expectation of that practice. Alternatively, the annual visit can also correspond to the administration of non-core vaccines, as the DOI of these vaccines is typically one year (Day, Horzinek, & Schultz, 2015). The guidelines regarding catteries state that all cats entering such facilities should be vaccinated, with "core" vaccines, before or at the time of entry and if

funds permit should be repeated as suggested in the guidelines toward individual animals (Day, Horzinek, & Schultz, 2015). Another important topic for VGG is the reporting of adverse reactions through the relevant reporting systems, thus the practice of pharmacovigilance. While they are aware that surveillance systems have significant differences, including in terms of development, in countries around the world, veterinarians should be encouraged whenever possible to report all possible adverse reactions to the manufacturer and the competent authority to expand the knowledge base that drives manufacturers to increase vaccine safety levels.

"We should aim to vaccinate every animal with core vaccines. Non-core vaccines should be given no more frequently than is deemed necessary" (Day, Horzinek, & Schultz, 2015)

The update published in 2015 places greater emphasis on the importance of demonstrating an EBVM approach to vaccination through the development of a new previously reported scheme and a more comprehensive bibliography; there have been changes in recommendations regarding the timing of core vaccinations in kittens to take into account new data on MDA persistence. Specifically, the last vaccination of the first vaccination series was moved to 16 weeks of age or older to give the option of reducing the vaccine booster interval from 12 months to 6 months of age. In addition, clarifications on intervals for vaccine recalls of adults who have received doses of Modified Live Virus (MLV) against FHV-1 and FCV were indicated. Finally, information on new vaccines such as the FCV vaccine containing two viral strains was incorporated. Furthermore, the reclassification of the Feline Immunodeficiency Virus (FIV) vaccine from "not recommended" to "non-core". A discussion of the use of

serologic testing with outpatient kits for antibody titration and applying these methods for outbreak management in kittens. Critical to feline medicine: an analysis of the most appropriate anatomical site for cat vaccination and an update of the disease sheets and list of frequently asked questions (Day, Horzinek, & Schultz, 2015).

These WSAVA guidelines do not serve as rules applicable worldwide because it is impossible to draw up a set of guidelines that can be applied worldwide in an area such as vaccination. Furthermore, the WSAVA includes 80 member countries that involve significant differences between nations, not only economic but also regarding the presence or prevalence of infectious disease, the availability of a particular vaccine and the number of feline population of property and not (strays). In addition, differences also need to be taken into account concerning the veterinary profession, financial availability and attitude towards vaccination of individual clients. Therefore, it is up to individual veterinarians and national associations to study and adapt these guidelines to their specific situations in practice. The VGG also reports on an issue that has concerned many veterinary practitioners, namely the inconsistency between the recommendations provided in the guidelines and what is reported in the Summaries of Product Characteristics [SPC]. They fear that this will open the door to litigation should they decide to adopt the guideline recommendations (Day, Horzinek, & Schultz, 2015). The clear difference between a package insert and a guideline document has been clearly discussed by Thiry and Horzinek (2007) (Day, Horzinek, & Schultz, 2015).

The DOI reported on package inserts describes the minimum duration of the relative product. In general, the DOI is based on experimental scientific evidence and quantifies how long after vaccination an animal

remains protected from the disease; the DOI is determined through experimental infections with very virulent viruses. Therefore, it does not necessarily reflect the actual DOI of a vaccine but simply a minimum value. Core vaccines have recently been commercially approved that report a minimum DOI of three years (Day, Horzinek, & Schultz, 2015).

In many countries, most core MLV vaccines are now licensed for 3-year recalls in adult animals. In countries where this has not yet happened, it is either because the manufacturer has requested a change in product recommendations or because the relevant authorities have not yet allowed this change. The VGG urges that even the current DOI of three years should be considered a minimum, and it is likely that the valid DOI will be significantly longer for most of those vaccinated. Therefore, the veterinarian is justified in "off-label" use by practicing a three-year rather than annual booster even using vaccines reporting a minimum DOI of one year, subject to obtaining informed consent from the owner of the vaccinated animal. The VGG recommendations seek to provide a perspective that takes into account worldwide differences in pet ownership. In conclusion, veterinarians should feel comfortable vaccinating following the protocols provided but should also cross-check with local recommendations, if available.

1. HINTS OF VACCINOLOGY

1.1 Adaptive immunity

"The main aim of vaccination against infectious diseases is to stimulate host adaptive immune responses to counteract the infection. In contrast to innate immunity, recognition of foreign antigens by an adaptive immune system is highly specific" (Takahashi, 2003).

The first line of defense of vertebrates is innate immunity, consisting of chemical and physical barriers (such as skin and mucous membranes) and "specialized" cellular elements such as neutrophil granulocytes and macrophages. These cells have the function to phagocyte and eliminate the antigen. However, they are not specific, as they cannot target a particular substance rather than another (Farina & Scatozza, 2002).

The second line of defense is triggered by the first and is represented by adaptive immunity, whose mechanisms are highly specific (Farina & Scatozza, 2002). "Principally, adaptive immunity consists of humoral and cell-mediated immunity" (Germain, 1994).

Defense mechanisms occur in three successive stages:

1. Recognition: that is, distinguishing between what belongs to the organism, self, and what is extragenic, non-self. This ability is a characteristic of cells such as macrophages, monocytes, and lymphocytes. The action of lymphocytes is specific, that is, directed toward a single foreign entity. On the surface of all cells, there are molecular structures (including the most important, the Major Histocompatibility Complex) that differ both between different species and between the same species. When they come into contact with the antigen, these receptors can

recognize it as self or non-self, and to signal in case of non-self, then an external agent to be eliminated. This defense mechanism is valid with any microorganism that exposes an antigen (viruses and bacteria) read and identified by the immune system (Farina & Scatozza, 2002).

2. Processing: this phase involves the processing and "presentation" of the antigen to the lymphocytes. Generally, this phase is carried out by the cells that have recognized the antigens (macrophages). Thus, triggering the processes of antigen elimination (Farina & Scatozza, 2002).
3. Effector response: the effector mechanisms are humoral and cell-mediated. The humoral response consists of the production of antibodies by activated B lymphocytes, free to move through the bloodstream. The antibodies bind to the antigen and eliminate it. The cell-mediated response instead leads to the activation of T lymphocytes that directly or indirectly destroy the foreign substance. These two types of cellular responses continuously interact with each other forming a cellular network that controls and modulates the activity of the immune system as a whole (Farina & Scatozza, 2002).

1.1.1 Lymphoid organs and lymphocytes

Lymphocytes regulate adaptive immunity. Lymphocytes are round, highly mobile cells. They originate in the bone marrow, a hematopoietic organ that contains precursors of blood cells, including lymphocytes. The primary lymphatic organs are the organs that regulate lymphocyte development. Lymphocytes are divided into two groups, T lymphocytes and B lymphocytes. The letter that characterizes their name is the initial of the name of the organ where

they mature. T lymphocytes mature in the thymus while the primary organ of B lymphocytes changes according to the species: in most mammals, they mature in the bone marrow; in birds, this phase is located in the “Bursa of Fabricius” (Tizard, 2013).

Lymphocytes must be able both to recognize the widest range of antigens that animals may encounter and to be able to find target cells within the body. This is made possible by the secondary lymphoid organs, which are widely distributed throughout the body. Lymphocytes, upon maturation, move from primary to secondary organs, whose anatomical structure facilitates antigen capture and provides the optimal environment for the initiation of an immune response. Within these organs are dendritic cells to trap and process antigens and lymphocytes to regulate the immune response. Examples of secondary lymphoid organs include the spleen, lymph nodes, tonsils, intestinal, respiratory, and urogenital lymphoid tissue. The presence of the antigen in the body stimulates the enlargement of these organs (Tizard, 2013).

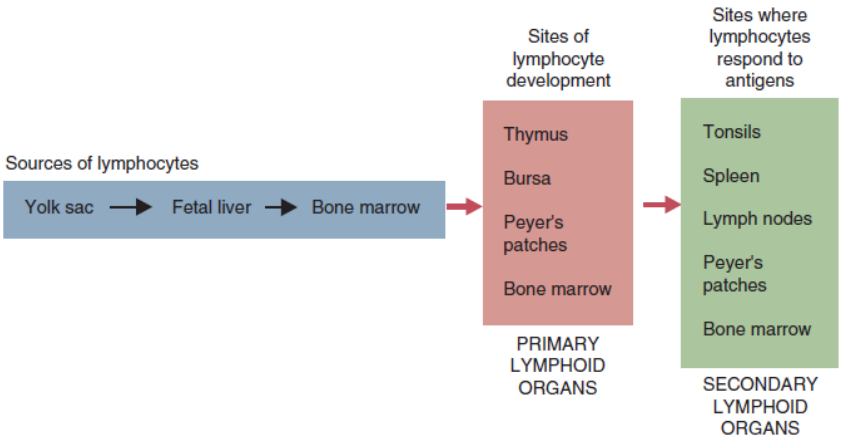

Figure 1 The lymphoid organs can conveniently be divided into three groups based on their role in the development and functioning of lymphocyte populations. (Tizard, 2013)

1.1.2 Humoral immunity

When an antigen enters an organism, it is usually phagocytosed by macrophages and processed. It is then exposed on its surface bound to the major histocompatibility complex class II. In this way, the antigen is recognized by B lymphocytes through their specific receptors: membrane immunoglobulins. Alternatively, the lymphocyte can come into direct contact with the antigen through its membrane receptors. The contact between the antigen and the lymphocyte stimulates the proliferation of lymphocytes that differentiate into two cell populations: plasma cells and memory cells. The first ones can secrete large amounts of antibodies, while the second ones constitute the immune memory. When the antigen for which they are specific will enter the body a second time, they will produce specific antibodies rapidly. When a B lymphocyte meets for the first time an antigen, it produces IgM, and only later IgG will be produced. Antibodies have neutralizing, agglutinating, precipitating, opsonizing, and finally complement fixation activities (Tizard, 2013).

1.1.3 Cell-mediated immunity

T lymphocytes regulate Cell-mediated immunity. T lymphocytes, following antigen binding, are activated and proliferate, producing more T cells. Among these cells, there are three subgroups: effector cells, regulatory cells, and memory cells. Effector cells are cytotoxic T lymphocytes, and their function is to directly attack target cells and cause their lysis through membrane alterations. Some cytotoxic T lymphocytes instead attract other cells such as cytokines and macrophages. Regulatory cells are T helper and T suppressor lymphocytes; the former activates macrophages while the latter limit

the antibody response to avoid damage to the body. Memory cells persist for long periods even after antigen elimination (Tizard, 2013).

1.2 Type of vaccines

An animal can be immune to an infectious disease in two ways: passive immunization or active immunization (Tizard, 2013). Passive immunization occurs with the transfer of antibodies from a resistant animal to a susceptible one. This results in the establishment of temporary but immediate immunity. The antibodies are gradually catabolized, protection wanes, and the animal becomes susceptible again.

In contrast, active immunization allows the establishment of an immune response through the administration of an antigen. Faced with a second exposure of the same antigen to the same animal the immune response will be significantly enhanced. The disadvantage of active immunization is that the protection is not immediate (Tizard, 2013). The benefit of active versus passive immunization includes the significantly prolonged period of protection and the greatly enhanced immune response due to actions such as vaccine booster, which involves repeated exposure to the antigen or infection (Tizard, 2013). An ideal vaccine should confer prolonged immunity to the vaccinated animal and the fetus carried by the animal. A vaccine should be able to stimulate adaptive immunity without triggering the inflammatory process associated with innate immunity. It should therefore be able to confer protection without side effects (Tizard, 2013).

“The ideal vaccine should be cheap, stable, and adaptable to mass vaccination; ideally, it should stimulate an immune response distinguishable from that due to natural infection so that immunization and eradication may proceed simultaneously.” (Tizard, 2013).

Vaccines can be distinguished as “infectious” or “non-infectious”. Infectious vaccines contain a live organism that is attenuated to reduce virulence. Thus, they are called “Modified Live Virus” or attenuated vaccines. The organisms in these vaccines are intact and viable and induce immunity by replicating within the animal and inducing low-level infection without producing tissue injury or clinical signs of infectious disease. Some recombinant vectored vaccines may also be considered “infectious”, a live vector organism carrying genetic material encoding an antigen from the target pathogen; however, the vector organism is not relevant to or pathogenic in the cat.

Non-infectious vaccines, also called inactivated or killed vaccines, including subunit and naked DNA vaccines, contain inactivated but antigenically intact pathogen or a natural or synthetic antigen derived from that pathogen. They are unable to infect, replicate or induce clinical signs of infectious disease. They need an adjuvant to increase their potency and multiple doses to induce protection (in puppies and adult animals). Non-infectious vaccines have a shorter Duration Of Immunity (DOI) than infectious vaccines and may be less likely to induce cell-mediated and humoral immunity (Day, Horzinek, & Schultz, 2015).

1.2.1 Living vaccines

Two of the main characteristics of the ideal vaccine are often incompatible: high antigenicity and no side effects. Modified Live Vaccines infect host cells and replicate within them. The infected cells process the endogenous antigen triggering a cell-mediated response dominated by cytotoxic T cells and Th1 cells. This can be dangerous because the viruses contained in the vaccine can cause diseases or persistent infections (residual virulence) (Tizard, 2013).

To minimize side effects in live vaccines, the virulence of the viruses is reduced so that even if they are still alive, they cannot cause disease. *Virulence reduction* is a process called attenuation. The attenuation level is a critical point in determining the success of a vaccine: an under-attenuation will result in residue virulence and consequently disease, while an over-attenuation can result in an effective vaccine (Tizard, 2013).

Traditionally, viruses were attenuated by growing them in cells or species that were not naturally adapted. For example, the Fury strain of rabies was attenuated by making passages in eggs and lost its virulence to dogs and cats. Another traditional method of attenuating viruses is to grow them on tissue cultures by growing the organism in cells that are not suitable for its full development. For example, the canine distemper virus has a tropism for lymphoid tissues and was grown in canine kidney cells. By adapting to those growing conditions, it lost its ability to cause severe disease (Tizard, 2013).

An ideal attenuation must make a virus avirulent without affecting its ability to activate an immune response. Finally, the attenuation must be independent of the host immune system, and the strain must be easy to grow, store and administer (Shams, 2005).

1.2.2 Inactivated vaccines

An inactivated vaccine (or killed vaccines) contains a virus rendered non-infectious by chemical processes and an adjuvant to amplify the restorative power of the immune response. To prepare an effective inactivated vaccine, it is necessary to consider two parameters: the amount of antigen and the composition of the adjuvant (Van Oirschot, 2001).

Killed pathogens result in the immune system as exogenous antigens (unlike live attenuated pathogens, which are instead recognized as endogenous antigens and invoke a cytotoxic response) and stimulate an immune response dominated by T helper cells type 2. One of the advantages of killed vaccines is that they induce an immune response that is safer for the body (Tizard, 2013).

The disadvantages are in line with the disadvantages of live vaccines; repeated exposure to the antigen through the various administrations can cause hypersensitivity reactions. Also, the use of adjuvants to increase efficacy can lead to severe inflammatory conditions or systemic toxicity (Tizard, 2013).

It is fundamental not to compromise the efficacy of a vaccine that the inactivated microorganism remains structurally similar to the live microorganism. For this reason, methods such as protein denaturation are often ineffective because they cause extensive changes in the structure of the antigen. If chemical processes are used, they must not alter the antigenic structure to stimulate protective immunity. Formaldehyde, for example, cross-binds proteins and nucleic acids and confers structural rigidity. Mild denaturation can be performed by acetone or alcohol. Alkylating agents can also help kill an organism. They leave the surface proteins unchanged, thus not interfering with antigenicity. Examples of alkylating agents used in veterinary medicine are acetyl ethylenimine and propiolactone. Many vaccines found to be effective contain killed bacteria (bacterins) or inactivated toxins using these alkylating agents (Tizard, 2013).

1.2.3 Modern vaccine technology

Continuous innovation in vaccines is essential to make them increasingly effective, affordable, and, most importantly, safe.

The use of modern technology can lead to new and improved vaccines (Tizard, 2013).

These vaccines fall into four categories:

1. Vaccines that contain recombinant inactivated organisms or purified antigens derived from recombinant organisms: the gene encoding for the viral antigen of interest is cloned into another organism and expressed and produced in large quantities. The first recombinant veterinary vaccine to be set up is against the Feline Leukemia Virus. The antigen being used is an envelope glycoprotein (gp70) that stimulates a protective immune response in cats. The gene that encodes for this protein was isolated and inserted into *E. coli*, along with a small portion of another protein that works in association with gp70. *E. coli* synthesizes p70, the same non-glycosylated protein, in enormous quantities. Once cloned, the recombined protein is purified, added to an adjuvant, and used as a vaccine (Tizard, 2013).
2. Vaccines containing live organisms containing gene deletions or heterologous marker genes: attenuation by tissue culture can be considered a primitive form of genetic engineering. The result, already explained in the previous paragraph, is developing a strain that cannot cause the disease. To this category belong DIVA vaccines (Differentiated Infected from Vaccinated Animals), the microorganism genes that serological techniques

for diagnostics can detect can be removed, ensuring the distinction between vaccinated animals and naturally infected animals (Tizard, 2013).

3. Vaccines that contain live expression vectors expressing heterologous genes for immunizing antigens or other stimulants: genes encoding for antigens can be cloned directly into other organisms instead of being purified, the recombinant organism can be used as a vaccine (Tizard, 2013).
4. Other genetically modified vaccines, such as polynucleotide vaccines: this category involves the use of DNA that encodes for an antigen, rather than using the protein antigen directly. The plasmid of a bacterium can be used as a vector. When the genetically modified plasmid is injected into an animal, it will be taken up by the host cells. Subsequently, the DNA will be transcribed into messenger RNA and translated into an endogenous protein to be vaccinated against. The safety of these vaccines is given because the plasmid cannot replicate inside mammalian cells. Adjuvants can be added to amplify the effect. (Tizard, 2013).

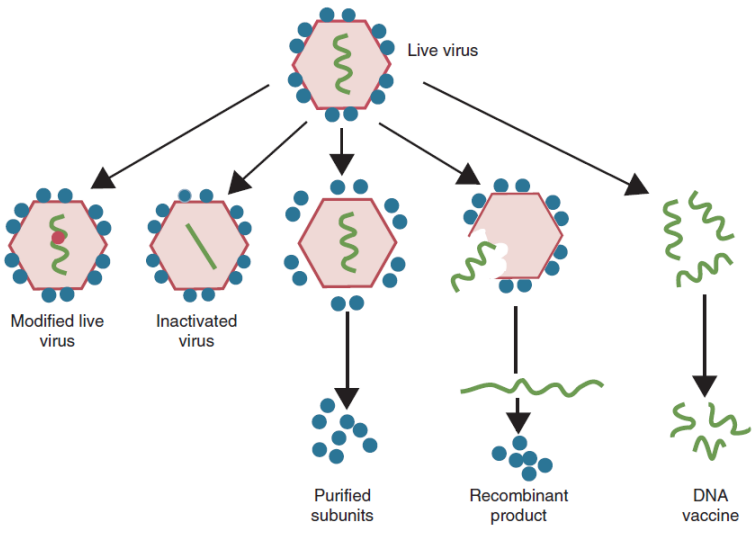

Figure 2 A schematic diagram showing some of the different ways in which a virus and its antigens may be treated in order to produce a vaccine. (Tizard,2013)

1.3 Adjuvants

Adjuvants (from the Latin verb “adjuvare”, “to help”) are substances capable of optimizing the effectiveness of a vaccine. They are mainly used in killed vaccines, where the killed organism has poor antigenic properties. Adjuvants can increase the speed of response to the vaccine or increase the intensity of the immune response. They can decrease the number of doses needed for the vaccine or a smaller amount of antigen. They are also essential for improving the duration of long-lived immunity. The mechanism of action is poorly understood, but they can be classified into three groups based on how they act. The first group, depot adjuvants, prolong the immune response by protecting antigens from degradation. The second group comprises particles that behave like dendritic cells by presenting the antigen to the cells of competence, the B and T lymphocytes. The third group is composed of immunostimulant adjuvants, i.e., molecules that increase the production of cytokines and the proliferation of Th1 and Th2 cells providing an additional stimulus to the immune response. Some act directly on B and T cells by increasing their proliferation or conversion into memory cells. They can optimize the immune response toward a specific antigen, so the choice of an adjuvant can influence the nature of the immunity cells produced and significantly increase the efficacy of a vaccine. Some common adjuvants are Aluminum phosphate or Alum (allow slow release of antigen), Lipopolysaccharide (stimulates macrophages), Saponin (stimulates antigen processing) (Tizard, 2013).

Table 1. Some common adjuvants. (Tizard, 2013)

TYPE	ADJUVANT	MODE OF ACTION
Depot adjuvants	Aluminum phosphate	Slow-release antigen depot
	Aluminum hydroxide	Slow-release antigen depot
	Alum	Activate DAMPs
	Freund's incomplete adjuvant	Slow antigen release depot
Microbial adjuvants	Anaerobic corynebacteria	Macrophage stimulator
	BCG	Macrophage stimulator
	Muramyl dipeptide	Macrophage stimulator
	Bordetella pertussis	Lymphocyte stimulator
	Lipopolysaccharide	Macrophage stimulator
Immune stimulators	Saponin	Stimulates antigen processing
	Lysolecithin	Stimulates antigen processing
	Pluronic detergents	Stimulates antigen processing
	Acemannan	Macrophage stimulator
	Glucans	Macrophage stimulator
	Dextran sulfate	Macrophage stimulator
Delivery systems	Liposomes	Stimulates antigen processing
	ISCOMS	Stimulates antigen processing
	Microparticles	Stimulates antigen processing
Mixed adjuvants	Freund's complete adjuvant	Depot plus immune stimulant

2. FELINE VACCINATION

2.1 Main infectious diseases

2.1.1 Feline Panleukopenia Virus (FPV)

Feline panleukopenia it is a viral infectious disease typical of domestic cats, characterized by high contagiousness and high mortality in kittens or young subjects. Mortality in kittens is over 90% (Truyen, et al., 2009). The predominant clinical signs are severe leukopenia and enteritis. Hence it takes the following names: feline panleukopenia or feline transmissible gastroenteritis. Prenatal infection, occurring about halfway through gestation, causes cerebellar hypoplasia in kittens born at term (Farina & Scatozza, 2002).

It 'a disease widespread worldwide, highly contagious. In addition to the domestic cat, it also affects minks, raccoons and foxes (Truyen, et al., 2009).

This pathogen can survive for long periods in the outdoor environment and is resistant to some detergents (Truyen, et al., 2009).

The transmission is fecal-oral route, with the most important source of infection being the feces of acutely infected animals (Farina & Scatozza, 2002). The course of this disease is cyclical, affecting unvaccinated animals mainly in kennels, feline colonies or farms. In places where the population density is high, receptive subjects are present, and vaccine prophylaxis is not adequately performed (Farina & Scatozza, 2002).

A diagnosis of suspicion can be made through observation of the clinical picture and find confirmation at necropsy. Laboratory examinations consist of the direct research of the antigen in feces or using marked antibodies in organs of deceased animals such as lymph

nodes or intestinal mucosa (Farina & Scatozza, 2002). Serological tests are not recommended because they do not distinguish the vaccinated subject from the infected subject (Truyen, et al., 2009).

Passive colostral immunity persists for approximately 6-14 weeks (Farina & Scatozza, 2002). Passive immunity protects against spontaneous infection but interferes with the immunogenic activity of the vaccine. Therefore, it is not recommended to vaccinate subjects before four months of age (Day, Horzinek, & Schultz, 2015).

2.1.1.1 Vaccines

For FPV, there are commercially available MLV vaccines for parenteral use and adjuvanted and non-adjuvanted quenched vaccines, also for parenteral use (Day, Horzinek, & Schultz, 2015). There is an intranasal non-adjuvanted MLV vaccine in combination with other antigens (FHV-1 and FCV) (Day, Horzinek, & Schultz, 2015).

MLV vaccines have the advantage of faster onset, greater efficacy in overcoming maternal antibodies, and a greater likelihood of conferring sufficient immunity (DiGangi, Levy, & Griffin, 2012).

All cats, including those that do not leave the house, should be vaccinated (Truyen, et al., 2009).

Guidelines call for initial vaccination at 6-8 weeks of age, then every 2-4 weeks until 16 weeks of age or older. (Day, Horzinek, & Schultz, 2015). Then do the booster at 6-12 months after the first vaccination cycle and every three years after that.

2.1.1.2 Duration Of Immunity

Following natural infection, DOI persists throughout life (Day, Horzinek, & Schultz, 2015). Experimental studies confirm that the following vaccination with inactivated vaccines, the duration of

immunity is approximately 7.5 years (Scott & Geissinger, 1999). With MLV vaccines, the DOI is approximately seven years (Day, Horzinek, & Schultz, 2015).

Maternal immunity confers protection from field infections but interferes with the immunity conferred by the vaccine. There is a time interval in which maternal immunity decays. Therefore, the pup is vulnerable to field infections, and the vaccine is not effective because the maternal antibody titer is still high enough to interfere. This situation is referred to as an immune gap (Day, Horzinek, & Schultz, 2015).

After the first vaccine cycle is completed, a booster is recommended at 6-12 months of age and then every three years, no more (Day, Horzinek, & Schultz, 2015). The presence of serum antibodies, regardless of titer, in an actively immunized cat more than 20 weeks old indicates protection (Day, Horzinek, & Schultz, 2015).

When vaccination is used to control disease in an outbreak in a cattery, the faster induction of immunity induced by MLV vaccines has a clinical advantage (Day, Horzinek, & Schultz, 2015). There is a very early onset of protection after vaccination with MLV products (Brun, Chappuis, & Precausta, 1979).

2.1.1.3 Advice

Intranasal combination FPV vaccines should not be used in kittens or, if used for immunity against FCV/FHV-1, should be administered concurrently with a parenteral MLV product for FPV (Schultz, 2009). MLV vaccines should not be used during cat pregnancy in FIV- and FeLV-positive individuals and puppies under 4-6 weeks of age (Day, Horzinek, & Schultz, 2015). Inactivated vaccines may benefit wild and

exotic species, pregnant cats, or retrovirus-infected cats in whom MLV vaccines are not recommended (Day, Horzinek, & Schultz, 2015).

2.1.2 Feline viral rhinotracheitis (FVR)

Viral infectious rhinotracheitis of cats is caused by Feline Herpes Virus type 1 (FHV-1). The disease has an acute course, and there are several clinical pictures. Severe symptoms of the upper airways characterize the most frequent picture; the clinical signs are nasal discharge, conjunctivitis, and cough. However, pneumonia and ulceration of the oral cavity and skin are also possible.

The disease is widespread worldwide and is believed to be the leading cause of respiratory disease in cats (Farina & Scatozza, 2002).

In most cases of FHV1 positive cats, the virus in question remains latent in the body of the host. The cat, in this case, becomes a lifelong carrier, and the use of corticosteroids or exposure to severe stress leads to the reactivation of the virus (Gaskell, Dawson, Radford, & Thiry, 2007).

It affects individuals of all races, sexes, and ages. It is widely distributed in warm areas and in places where cats are retired or housed. Outbreaks of the disease are periodically observed in feline colonies and wherever there is a high density of feline population, either by the entry of susceptible subjects to infection or by the reactivation of viruses in a latent state. The severity of outbreaks is determined by population immunity (Farina & Scatozza, 2002).

It is a labile virus in the external environment; the main transmission route is direct contact through nasal secretions of infected animals. In case of reactivation, the excreted portion of the virus is of lower amounts, and direct contacts must be prolonged.

The infected mother can transmit the virus to the newborn, who will not show clinical signs because he is covered by MDA but will still undergo

a state of infection and then latency. This mechanism can also occur following immunization prophylaxis (Farina & Scatozza, 2002).

Diagnosis can be made with PCR on conjunctival or oropharyngeal swabs. biopsies or corneal scrapings (Thiry, et al., 2009).

2.1.2.1 Vaccines

Adjuvant-free MLV vaccines containing a single serotype of FHV attenuated to various titers are available. The route of administration is parenteral; there are monovalent or polyvalent preparations where FHV is combined with FCV and FPV. Inactivated and adjuvanted vaccines are also available (Day, Horzinek, & Schultz, 2015).

According to WSAVA guides, the first administration should be done at 6-8 weeks of age, then every 2-4 weeks until 16 weeks of age. For adults with unknown vaccine prophylaxis, two doses are recommended 2-4 weeks apart. The booster should be carried out at 6 or 12 months of age, then every three years for low-risk cats (indoor cats). For high-risk cats (e.g., colony cats or cats that leave home), annual revaccination is recommended (Day, Horzinek, & Schultz, 2015).

2.1.2.2 Duration Of Immunity

The protection provided by the vaccine for FHV-1 is not as durable as that provided by the vaccine for FPV. Assessing DOI is difficult. Complete clinical protection occurs only a short time after vaccination, and the degree of protection decreases with time (Gaskell, Dawson, Radford, & Thiry, 2007). After natural infection, immunity is of variable duration. The persistence of antibody titers after vaccination with an inactivated FHV-1 vaccine has been shown to be three years (Scott & Geissinger, 1999), but antibody titer for FHV-1 does not correlate well with protection (Gaskell, Dawson, Radford, & Thiry, 2007).

After the first round of vaccines at 16 weeks or more, a booster should be performed at 26 or 52 weeks and every three years thereafter in low-risk cats.

High-risk cats (particularly cats in shelter) should be revaccinated more often. If vaccine booster shots have expired in a previously regularly vaccinated cat, a single dose may be sufficient to recall immune memory (Day, Horzinek, & Schultz, 2015). No vaccine for FHV-1 can protect against infection with virulent viruses. The virus can go into latency and reactivate after periods of intense stress, even in vaccinated animals (Gaskell, Dawson, Radford, & Thiry, 2007) MDAs interfere with vaccine action. In feline herds, the most affected are the kittens in the weaning period when MDAs decrease, and the source of infection is the cat in which the virus has reactivated due to lactation stress (Day, Horzinek, & Schultz, 2015).

2.1.2.3 Advice

Parenteral MLV vaccines for FHV-1 and FCV may induce disease if it is incorrectly administered because they retain pathogenic potential. Side effects may occur if accidentally ingested or inhaled (Day, Horzinek, & Schultz, 2015). Clinical signs of disease have occurred following intranasal administration (Day, Horzinek, & Schultz, 2015).

2.1.3 Feline Calicivirus (FCV)

Feline calicivirus infects the upper airways extending to the lungs, causing disease with an acute or sub-acute course (Farina & Scatozza, 2002).

The disease is present worldwide and is frequent in cats between 1 and 12 months of age. It is prevalent in places where the concentration of cats is high such as breeding farms, veterinary clinics, and feline colonies (Farina & Scatozza, 2002). The prevalence of the disease is high; more than 90% of cats have specific antibodies (Farina & Scatozza, 2002).

The infection occurs through direct contact; the excretion of the virus occurs orally, hardly through feces. Some subjects can become carriers even after recovery. The virus persists for long periods in the tonsils and is excreted continuously. Carriers have modest titers of neutralizing antibodies (Farina & Scatozza, 2002).

Characteristic symptoms are ulcers of the oral cavity, respiratory symptoms, and high fever (Radford, et al., 2011). Pictures of chronic stomatitis or gingivitis are also present; the virus can be isolated from these lesions. If the virus is particularly virulent, affected individuals show lesions such as skin edema, ulcers on the head and limbs, and jaundice (Radford, et al., 2011).

Mortality is high, and the disease is more severe in adults (Radford, et al., 2011). Diagnosis can be made from the previously listed clinical symptoms and confirmed by immunofluorescence or following seroconversion. Vaccines, often associated with FHV-1 and FPV, are available to prevent infection.

2.1.3.1 Vaccines

Non-adjuvanted MLV vaccines are available for parenteral and intranasal use. Non-adjuvanted inactivated vaccines containing two strains of calicivirus (strains G1 and 431) (Poulet, Brunet, & Leroy, 2005). One adjuvanted inactivated vaccine for parenteral use.

Prophylaxis should be started at 6-8 weeks of age, then every 2-4 weeks until 16 weeks or more (Day, Horzinek, & Schultz, 2015). In adults with unknown vaccine prophylaxis, two doses 2-4 weeks apart are recommended. The booster should be done at six months or one year of age and then every three years for indoor cats and every year for cats at high risk of exposure (Day, Horzinek, & Schultz, 2015).

2.1.3.2 Duration Of Immunity

Neutralizing antibodies appear approximately seven days post-infection. Local secretory IgA and cell-mediated immunity protect vaccinated individuals, and this allows the cat to be protected even in the absence of serum antibodies (Day, Horzinek, & Schultz, 2015).

Experimental studies have shown the persistence of antibodies for at least four years following vaccination with adjuvanted inactivated vaccine (Scott & Geissinger, 1999). The immunity conferred by experimental infection with virulent FCV 7.5 years after vaccination with two doses of inactivated adjuvanted vaccine was like the protection after one year with the inactivated product (Scott & Geissinger, 1999).

The protection provided by FCV vaccines cannot be considered on a par with that provided by other core vaccines (FHV-1 and FPV), is incomplete, and reinfection with other strains of the same virus cannot be excluded. Moreover, it does not provide the same degree of protection and the same duration as the other core vaccines.

Therefore, it is advisable to vaccinate cats at high risk of exposure at intervals shorter than three years (Day, Horzinek, & Schultz, 2015).

For puppies, it is recommended to always use the same strains during the first round of vaccination. MDAs can interfere with vaccine action and have a half-life of about 15 days with persistence up to 14 weeks (Johnson & Povey, 1983). MDAs interfere primarily with MLV vaccines and less so with intranasal vaccines. Puppies given IN vaccination become immunized earlier than puppies vaccinated with MLV (Day, Horzinek, & Schultz, 2015).

2.1.3.3 Advice

Upper respiratory tract problems have been reported following IN administration (Lappin, Sebring, & Porter, 2006). Despite the choice to include different viral strains in vaccines to increase the likelihood of cross-protection, the high antigenic variability of the virus does not preclude the possibility of mild clinical manifestations in vaccinated individuals (Day, Horzinek, & Schultz, 2015). Unlike FHV-1, which is excreted only after stressful events, FCV dissemination is continuous but generally ceases after many months (Coyne et al. 2006a).

"The impact of vaccination on excretion is controversial, with observations ranging from a moderate reduction to an extension of the period of post-infection viral excretion. Live parenteral FCV vaccine strains may be excreted, although not frequently." (Day, Horzinek, & Schultz, 2015).

2.1.4 Rabies

Rabies is an infectious disease transmitted by a virus of the genus *Lyssavirus* of the family *Rhabdoviridae*.

There are different rabies virus genotypes with specific reservoirs. Infection is transmitted only through rabid animals by biting or scratching skin or mucous membranes.

The virus is located at the level of salivary glands, and the animal can be infectious even before the manifestation of clinical symptoms.

The incubation period is highly variable (from two weeks to many months); in the cat, on average is two months.

A sudden alteration in behavior and sudden aggression by the cat should be considered suspicious. Two clinical forms are present in cats: the "mute" form and the "furious" form. Clinical signs of the most common form for cats, the "furious" form, include reduced eyelid and eye reflexes, squinting, drooping jaw, sialorrhea, tremors and convulsions, disorientation, irritability, unmotivated anger and fear, photophobia, followed by paralysis, coma and death from respiratory arrest.

The prognosis is unlucky, and death occurs 1-10 days after the first clinical manifestation.

The diagnosis of certainty is possible only with post-mortem laboratory analysis directly on the Central Nervous System (CNS) through techniques such as direct immunofluorescence (FAT) and virus isolation in cell culture (RTCIT) or RT-PCR as confirmatory tests.

Post-exposure vaccination is prohibited in many countries and changes according to public health regulations from nation to nation (Frymus, et al., 2009).

Vaccinations are recommended in geographic areas where rabies is endemic and are mandatory if there is a mobilization of pets from one nation to another. Kittens should not be vaccinated before 12-16 weeks of age to avoid interference with MDA (Day, Horzinek, & Schultz, 2015).

2.1.4.1 Vaccines

There are three types of vaccines available for Rabies:

1. MLV: whose use is banned in the EU is mainly used for oral immunization of wild animals, derived from the viral strain Street Alabama Dufferin (SAD).

2. Vaccines with recombinant vector: in Italy, the vaccine vector used for a recombinant vaccine in cats is the canarypox virus, containing a gene of the rabies virus that encodes for the G-glycoprotein necessary for protection. It is not adjuvanted. Other types of vectors used in other countries are adenoviruses and poxviruses. These vaccines are avirulent in all avian and mammalian species tested.

3. Inactivated (switched off) vaccines: In the EU, they are the only vaccines authorized together with recombinant vectors as they are easier to manage than MLV vaccines because they are stable at room temperature and do not represent a risk in case of accidental self-inoculation.

The vaccination protocol involves a single administration at 12 weeks of age in the puppy with revaccination one year later. In the previously unvaccinated adult, a single administration and revaccination one year later is required. Revaccination should be managed according to the DOI listed on the vaccine package insert or according to local

regulations. The rabies vaccine is considered core only in areas where the disease is endemic (Day, Horzinek, & Schultz, 2015).

2.1.4.2 Duration Of Immunity

Although mainly switched-off vaccines are used for rabies control in feline and canine populations, recombinant vector vaccine is also used in cats in the EU and the USA because it is not associated with the inflammation at the inoculation site caused by adjuvanted rabies vaccines (Day, Horzinek, & Schultz, 2015).

All initial administrations must be followed by revaccination the following year to extend the vaccination interval to 3 years (Day, Horzinek, & Schultz, 2015).

It is impossible to calculate DOI after natural infection because the prognosis for "street" infection is always unlucky. DOI for inactivated and recombinant products is three years, established following experimental infections and serologic studies (Jas, Coupier, & Edlund Toulemonde, 2012).

The presence of serum antibodies at a titer ≥of 0.5 IU/ml in a dog more than 16 weeks old that has been actively immunized is an index of protection. Achieving this concentration (≥0.5 IU/ml) is also considered a legal requirement for pet movement in some countries that require post-vaccination serologic testing in their animal movement protocol (Day, Horzinek, & Schultz, 2015).

The VGG urges all legislators to consider scientific advances when formulating regulations. Some vaccines (e.g., nationally produced vaccines) may not reliably protect for more than one year. In the EU, the rules for handling and DOI of rabies vaccine are expressed in EU Regulation No. 576/2013 (Day, Horzinek, & Schultz, 2015).

2.1.5 Feline Leukemia Virus (FeLV)

Feline leukaemia virus (FeLV) is a type C *retrovirus*.

The infected cat presents with neoplastic pictures or other symptoms such as anemia, glomerulonephritis and panleukopenia (Farina & Scatozza, 2002). The most common symptoms indicating persistent viremia are immune system suppression, anemia, and lymphoma. Less commonly, chronic enteritis, reproductive disorders, and peripheral neuropathy can be seen in infected cats. The subject with persistent viremia usually dies within 2-3 years (Lutz, et al., 2009).

The disease is widespread worldwide, with a higher incidence in purebred animals, males and young cats. It is found primarily in areas where socialization is high such as catteries or farms, where transmission is facilitated (Farina & Scatozza, 2002).

It can be transmitted vertically, trans placentally or by milk intake from a viremic mother. Or horizontally via excretion of urine, feces, or saliva. However, the virus is labile in the external environment and is sensitive to most disinfectants (Farina & Scatozza, 2002).

The diagnosis can be addressed based on clinical symptoms, radiographic examination in case of suspicion of neoplasms, hematochemical analysis to verify the status of organs involved such as kidneys and liver and hematocytological analysis to identify the type of anemia. In the laboratory, techniques such as isolation or direct or indirect enzyme immunoassays can be applied (Farina & Scatozza, 2002).

This virus requires strict prophylaxis because the contagiousness is high, and it is good practice to perform diagnostic tests to exclude the disease even in the absence of symptoms.

2.1.5.1 Vaccines

An unadjuvanted recombinant canarypox vaccine, an adjuvanted inactivated vaccine, and an adjuvanted recombinant protein subunit vaccine are available.

These are all non-core vaccines; only FeLV negative cats should be vaccinated, so serologic testing is required before vaccination.

If it is considered necessary to vaccinate for FeLV in the puppy must be performed the first administration, not before eight weeks of age and followed by a second administration after 3-4 weeks. In adults, two doses 3-4 weeks apart are necessary.

The booster should be done one year after the first cycle and then after 2-3 years (not less) and only in cats considered at high risk of exposure (Day, Horzinek, & Schultz, 2015).

2.1.5.2 Advice

It should be considered that annual revaccination with adjuvanted vaccines may increase the risk of sarcoma development at the inoculation site, so vaccinate only if strictly necessary. A cat positive for FIV or FeLV that is clinically healthy should ideally live indoors away from other cats to minimize the risk of exposure to infectious disease. These cats should not be vaccinated against FIV or FeLV. An FIV or FeLV positive cat with the clinically manifest disease should not be vaccinated. An FIV or FeLV positive but clinically healthy cat can be vaccinated with core vaccines if necessary (Day, Horzinek, & Schultz, 2015).

2.1.6 Feline Immunodeficiency Virus (FIV)

Feline immunodeficiency virus causes a widespread chronic disease worldwide with a higher incidence in whole male cats over 5-8 years of age (Farina & Scatozza, 2002).

Environments where promiscuity and straying are high favor transmission of this virus (Farina & Scatozza, 2002).

The virus is present in saliva and the inflammatory cells of gingival lesions typical of infection. Infection is transmitted by saliva or blood inoculation through bite wounds and occurs in 4 stages of varying duration, and sometimes seropositive animals appear healthy for several months or years (Farina & Scatozza, 2002).

FIV infection is often associated with FeLV infection. FIV-positive individuals result as a perennial source of infection because they are persistently viremic despite the ability to produce both a humoral and cell-mediated immune response (Hosie, et al., 2009).

Infected cats remain asymptomatic for long periods, usually years. Most symptoms are due to immunodeficiency and secondary pathogen infections. Typical symptoms include chronic stomatitis, rhinitis, lymphadenopathy, and immune-mediated glomerulonephritis associated with significant weight loss (Hosie, et al., 2009).

The standard gold test for FIV is western blot (Hosie, et al., 2009).

An FIV-positive cat can lead an everyday life as uninfected cats and should never be euthanized solely based on a positive test result. Infected cats should be sterilized to avoid transmission of the virus, which is typical of male cats fighting each other for territoriality and receive regular health care (Hosie, et al., 2009).

The basis of prophylaxis against this virus is the control of stray cats by monitoring and sterilising strays.

2.1.6.1 Vaccines

Only an inactivated, adjuvanted vaccine is available. To complete an effective vaccination cycle, three administrations are necessary: at two maximum three weeks apart. In puppies, the first administration should take place after eight weeks of age. Revaccination should be annual and only in cats in which the high risk of exposure is inevitable (Day, Horzinek, & Schultz, 2015).

2.1.6.2 Advice

An FIV-positive cat should not be vaccinated for FIV, so a rapid serology test should always be performed to check for serum antibodies before vaccination. In addition, a cat vaccinated for FIV must be microchipped (Day, Horzinek, & Schultz, 2015).

The current FIV vaccine contains examples of two subtypes of FIV (A and D), and although it is claimed that there is cross-protection with other subtypes, there are geographic differences in the viruses circulating in different countries (Day, Horzinek, & Schultz, 2015).

The European ABCD (Advisory Board on Cat Diseases) reiterates the lack of evidence regarding the efficacy of this vaccine against European isolates and does not recommend its use. Cats vaccinated for FIV may become infected and infect other susceptible cats as vaccination does not prevent infection or latency (Day, Horzinek, & Schultz, 2015).

2.1.7 Feline Infectious Peritonitis (FIP)

Feline infectious peritonitis is a highly contagious disease that is widespread worldwide. The etiologic agent is a feline coronavirus (Farina & Scatozza, 2002).

Feline coronavirus is ubiquitous in cats, most of which are healthy or have signs of mild enteritis. Some cats develop feline infectious peritonitis, mainly young cats living in contact with other cats are affected (Addie, et al., 2009).

Coronaviruses differ in virulence and infectivity; in fact, the clinical pictures can vary from mild enteritis to peritonitis. They have affected mainly cats from 6 months to 3-4 years (Farina & Scatozza, 2002).

There are still doubts about the route of transmission. It is assumed that the pathogen is taken by ingestion or inhalation. Enteritis-causing strains spread via the fecal-oral route. The transplacental route is also probable, explaining why there are often more cases in a single litter and subsequent litters (Farina & Scatozza, 2002).

The first clinical symptoms are non-specific: intermittent fever, loss of appetite, weight and liveliness.

Subsequently, it is possible to find two clinical forms: exudative form and dry form. The exudative form is characterized by the accumulation of intraperitoneal fluid, rich in fibrin and is then clearly visible an increase in abdominal volume and subsequent dyspnea. Other possible symptoms are vomiting, diarrhea and jaundice in the final stage.

The prognosis is generally inauspicious. In addition to the previously mentioned non-specific symptoms, the dry form involves mesial lymphadenopathy appreciable at palpation, nodular lesions in different

organs. There are neurological signs such as muscle rigidity, locomotor ataxia, and ocular lesions in the most severe cases. Also, in this case, the prognosis is inauspicious (Farina & Scatozza, 2002).

The diagnosis is made through the observation of clinical signs and the evaluation of the history. If present, an analysis of the abdominal effusion can be performed, which appears stringy and yellowish.

It is possible to perform serology by ELISA and indirect immunofluorescence. The diagnosis of enteric forms is more difficult than others because of the difficulty in isolating the responsible agent and the low sensitivity of diagnostic methods (Farina & Scatozza, 2002).There is no vaccine prophylaxis feasible, so the control of FIP is based primarily on hygiene prophylaxis (Farina & Scatozza, 2002).

2.1.7.1 Vaccines

There is only one non-adjuvanted MLV vaccine for intranasal use. It should not be administered before 16 weeks of age, and a second dose is needed after 3-4 weeks. The manufacturer recommends an annual booster. The VGG considers this vaccine "not recommended." Following the few studies available, only seronegative cats (no antibodies) for feline coronavirus are likely to develop some protection at the time of vaccination. It is rare for a cat to be seronegative (absence of antibodies) for coronavirus at 16 weeks or older (Day, Horzinek, & Schultz, 2015).

2.2 Feline vaccination guidelines

2.2.1 Core vaccines

"Core" vaccines are all vaccines that are strongly recommended regardless of geographic location or circumstances (Day, Horzinek, & Schultz, 2015).

According to the VGG, the entire feline population should be vaccinated with these vaccines. The VGG identifies the vaccines for FPV, FHV-1 and FCV as core vaccines. The Rabies vaccine can be considered core or non-core depending on which country rabies is endemic or not. If rabies is endemic, the VGG recommends vaccinating the entire feline population as a routine practice to ensure safety for both animal and public health. In addition, it is generally mandatory in every country to vaccinate for rabies when the cat is subject to movement from one state to another (Day, Horzinek, & Schultz, 2015).

It should be noted that cat core vaccines do not provide the exact duration of immunity or level of protection as dog core vaccines. In addition, the protection provided by the FHV-1 and FCV vaccines is much lower than the protection provided by the FPV vaccine (Day, Horzinek, & Schultz, 2015).

There is no vaccine for FHV-1 that protects against a highly virulent virus, and the infection produced by the vaccine may result in the virus becoming dormant and then reactivated during periods of high stress for the cat (Richter, Schudel, Tobler, & al., 2009) (Maes, 2012). This results in the animal exhibiting clinical signs of FHV-1 and infecting susceptible animals.

The FCV vaccine is set up to provide cross-protection between different strains of FCV. However, given the wide variety of strains of that virus present, it is possible that a vaccinated adult cat could become infected and manifest clinical signs of disease (Pedersen, Elliott, & Glasgow, 2000) (Schorr-Evans, Poland, Johnson, & Pedersen, 2003).

Studies by Scott & Geissinger (1999) showed that for both vaccines (FCV and FHv-1) the DOI is approximately 7.5 years post-vaccination. However, more recent studies have identified significantly less protection against FHV-1 three years after the last vaccination with an MLV vaccine, while the protection provided by an MLV vaccine for FCV remains like the previous study (Jas, Frances-Duvert, Vernes, 2015)

For these reasons, the VGG recommends that cats considered high risk be vaccinated annually for FHV-1 and FCV. "High risk" refers to cats that have access to the outdoors or are regularly boarded. For low-risk cats, cats that live indoors and are not in contact with other animals with unknown vaccine prophylaxis, vaccination for FHV-1 and FCV can be done every three years (Day, Horzinek, & Schultz, 2015).

2.2.2 Non-core vaccines

VGG defines "non-core" vaccines as those required for animals at risk of contracting certain infectious diseases based on their geographical location, environment, and lifestyle (Day, Horzinek, & Schultz, 2015).

Therefore, they are recommended or not based on the individual epidemiological data of each country and the risk to which the animal is exposed.

The VGG also recognizes some vaccines as "not recommended" when the risks regarding using those vaccines are more significant than the benefits or when there is no scientific evidence to justify their use (Day, Horzinek, & Schultz, 2015).

In the case of cats, the only vaccine classified as "not recommended" is the vaccine for FIP, while the vaccines considered "non-core" are for the following infectious diseases: FeLV, FIV, *Chlamydia Felis* and *Bordetella bronchiseptica* (Day, Horzinek, & Schultz, 2015).

It should be noted that the vaccine against the Feline Leukemia virus is currently the subject of debate among experts (Day, Horzinek, & Schultz, 2015).

The VGG promotes and supports risk performance: benefit analysis as a routine practice between veterinarian and client. The cat's lifestyle and the prevalence of the infection in the area in which one is located should be considered. In geographic areas where this disease is prevalent, the VGG recommends routine vaccination by one year of age, specifically the administration of two doses at 2-4 weeks apart, starting no earlier than eight weeks of age (Day, Horzinek, & Schultz, 2015).

Regarding the FIV vaccine, the VGG has recently reclassified it from "not recommended" to "non-core", recognizing its usefulness in areas with a high prevalence of the virus and where the cat's indoor lifestyle is not expected. The vaccine was effective in some studies but not in all is therefore recommended only in conditions of high risk for the cat (Day, Horzinek, & Schultz, 2015).

2.2.3 Kitten vaccination

MDAs confer immune protection to the puppy during the first weeks of life. However, it must be considered that MDA intake varies from subject to subject, even within the same litter. Without a serological test that determines an antibody titer, the level of protection and the likelihood that the subject will respond correctly to vaccination is unknown (Day, Horzinek, & Schultz, 2015).

In general, MDA disappears between 8 and 12 weeks of age and from that time, it will be possible to produce an active immune response (Day, Horzinek, & Schultz, 2015). However, there may be subjects with low titer of MDA that will be vulnerable and therefore able to respond to a vaccination earlier, while others may have a titer so high as to compromise the proper functioning of the vaccine, sometimes up to 12 weeks of age (Day, Horzinek, & Schultz, 2015). That is why the VGG analyzed studies from 2012 suggesting that up to a third of puppies may not respond to a core vaccination at 16 weeks of age (DiGangi, Levy, & Griffin, et al.,2012) (Jakel, Cusser, Hanschmann, & et al, 2012). Therefore, the VGG increased the recommended age for the last vaccination of the first core vaccination cycle from 14-16 weeks to 16 weeks or more (Day, Horzinek, & Schultz, 2015). However, it should be considered that the above studies analyzed a low number of animals, primarily purebred and in a farm setting. This suggests that the data provided may not be fully applicable to the entire feline population (Day, Horzinek, & Schultz, 2015).

The VGG, therefore, recommends starting with the first administration of core vaccines at 6-8 weeks of age and then repeating vaccinations every 2-4 weeks until 16 weeks of age or older. Therefore, following what has been said, if this protocol is followed, the total number of

core vaccines will be 4, while if the first one was given at nine weeks of age, only three would be sufficient.

Traditionally the booster is done at 12 months of age or 12 months after the last vaccination. The purpose is to ensure that if a cat fails to respond adequately to one of the three administrations can develop a protective immune response through this booster. So, the purpose is not so much to "recall" the immune system but to ensure potentially unestablished protection (Day, Horzinek, & Schultz, 2015). This implies that a kitten can potentially remain uncovered until the recall. This could explain cases of infectious disease in some kittens under 12 months of age that have been properly vaccinated. Therefore, the VGG recommends that this vaccination be brought forward to 26 weeks of age or no later than within a range of 26 to 52 weeks (Day, Horzinek, & Schultz, 2015). For core vaccines, subsequent vaccination will not be necessary for at least three years (indoor cats). This does not preclude the possibility of still having a routine annual visit at 12 months, which may be one of the reasons why booster shots are historically given in that time frame (Day, Horzinek, & Schultz, 2015).

2.2.4 Sites of vaccination

Traditionally, the site of inoculation of vaccines in cats is between the shoulder blades. Vaccines, however, are a class of injectables related to a specific pathology in cats: Feline Injection Site Sarcoma (FISS). The vaccines considered to be at most significant risk of leading to this disease are the adjuvanted FeLV and rabies vaccines (Kass, Barnes, Spangler, & et al., 1993).

Numerous studies have been performed on FISS, and there are recent articles on this topic (Martano, Morello, & Buracco, 2011) (Ladlow, 2013) (Hartmann, Day, Thiry, & et al., 2015).

The pathogenesis of FISS is unproven, but the most reliable hypothesis is that chronic localized inflammation due to vaccine inoculation results in malignant transformation of mesenchymal cells and that there is also a genetic basis for this process to occur. The most common site to find this type of neoplasm is between the scapulae, where the vaccine is given. The problem with the interscapular space is that the surgical methods required to remove such an invasive tumor would be inapplicable (Martano, Morello, & Buracco, 2011).

In North America, AAFP guidelines (Scherk, Ford, Gaskell, & et al., 2013) recommend vaccinating FeLV and Rabies in the most distal part of the left and right hind limb, respectively, and giving the three core vaccinations in the distal part of either forelimb.

One study evaluated the effect of this practice by comparing the anatomic distribution of FISS before this recommendation was made (1990-1996) and after the adoption of this practice (1997-2006) (Shaw, et al., 2009).

The data demonstrated decreased interscapular FISS and increased FISS solely in the right limb and regions combined with the related limbs such as the right and left abdominal regions. Involvement of the abdominal region was attributed to the difficulty of vaccinating on the limb resulting in accidental injection into the nearest region (Day, Horzinek, & Schultz, 2015).This practice has not been widely adopted outside of North America.

Recently, a publication demonstrated the efficacy of administering FPV and rabies vaccines in cats' tails (Hendricks, Levy, Tucker, & et al., 2014).

The tail may prove to be a safer inoculation site than the regions previously listed, but further studies are needed in this regard (Day, Horzinek, & Schultz, 2015).

The question of the best inoculation site for the cat remains a debated and controversial topic; the VGG recommends acting according to one's convenience. However, one must always consider that any risk of FISS is outweighed by the benefit of protective immunity conferred by vaccines. Current prevalence estimates of FISS are 1 case per 5,000-12,500 vaccinated cats (Gobar & Kass, 2002).

The VGG recommends that non-adjuvanted vaccines always be preferred; if adjuvanted products need to be used, vaccination should not be performed at the interscapular site (Day, Horzinek, & Schultz, 2015).

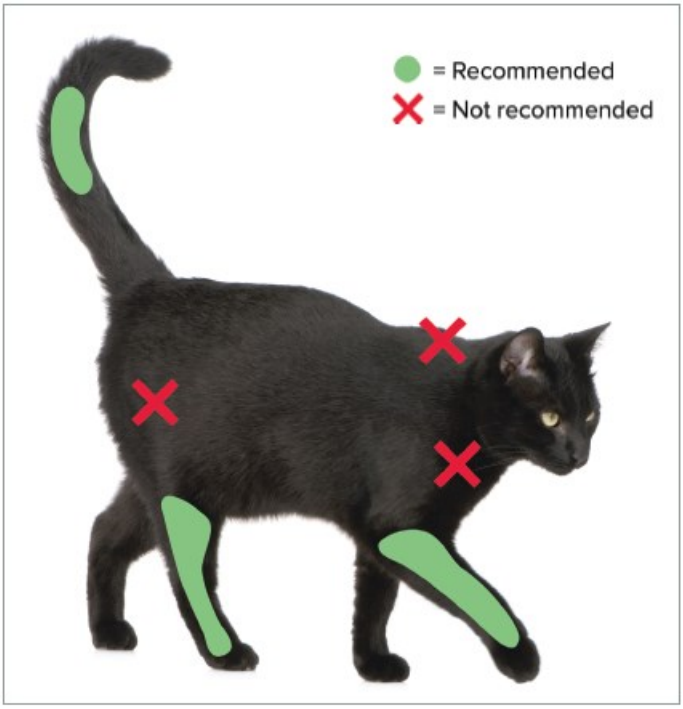

Figure 3 Vaccination sites: recommended injection sites in the distal limbs and tail. © iStock.com/GlobalP. (2020 AAHA/AAFP Feline Vaccination Guidelines)

One must always consider the possibility of vaccination in other subcutaneous sites (never muscular) depending on how complicated a possible surgical resection of a FISS in the chosen site can be. It is also recommended to register the inoculation site from time to time, perform an incubation site rotation plan, and report all suspected cases of FISS to the vaccine company and the appropriate pharmacovigilance systems (Day, Horzinek, & Schultz, 2015).

2.3 Duration Of Immunity

All that the scientific community has understood about the phenomenon of immunological memory is the persistence of memory cells, B cells, plasma cells, and T cells after vaccination. These cells allow for long-term protection. "The presence of long-lived plasma cells is associated with persistent antibody production so that a vaccinated animal may have antibodies in its bloodstream for many years after exposure to a vaccine. It is believed that these long-lived plasma cells are stimulated to survive by activation with microbial PAMPs acting through TLRs and that it is the antibodies that are mainly responsible for long-term protection.” (Tizard, 2013).

Vaccine schedules are established according to the duration of protection depends on the antigen contained, whether the vaccine consists of live or dead organisms and the route of administration. The most recent and modern vaccines require vaccination every three years, while for others, immunity may persist for the animal's lifetime. Generally, MLV vaccines confer a higher DOI than extinguished viral vaccines, although it is not excluded that they too may protect individual animals for many years. Unfortunately, reliable figures are not yet available for many vaccines because studies of the duration of immunity are a very recent practice. There are also not much data available on the DOI conferred by mucosal immunity (Tizard, 2013). Generally, immunity against FPV, thus feline panleukopenia, is relatively long-lasting as it is greater than five years. While for FCV, FHV-1 and Chlamidophila immunity is considered short. A significant problem with DOI studies is that there can be a significant difference between the shortest and longest DOI within a group of animals. In many studies, it has been found that older animals are the ones that

show more excellent innate resistance; this makes DOI studies incomplete (Tizard, 2013).

Another factor that makes it difficult to establish standard data for all vaccinated subjects is that vaccines even within the same category may differ in their composition, and although all may induce basic short-term immunity, it cannot be assumed that all will confer long-term immunity. Annual revaccination is an administratively straightforward approach and ensures that the veterinarian regularly sees an animal. "It is clear, however, that vaccines such as those against canine distemper or feline herpesvirus induce protective immunity that can last for many years and that annual revaccination using these vaccines is unnecessary." (Tizard, 2013).

MLV vaccines induce long-lasting, even lifelong, immunity. This is not the case with bacterial vaccines, whose duration is very short and often prevent disease onset but not the infection. Older dogs and cats rarely die from vaccine-preventable diseases, especially if they were vaccinated as adults. In contrast, young animals may die from missed or improper vaccination. A veterinarian should weigh the risks and benefits of using any vaccine and its frequency of administration and keep in mind that the duration of immunity claimed by a vaccine manufacturer is the minimum duration and not necessarily the actual duration. Serum antibody detection tests can be used to support this, although persistent antibody titers do not necessarily indicate protection, especially if the necessary protection is conferred by cell-mediated immunity. (Tizard, 2013)

2.3.1 Serological Testing to determine the Duration Of Immunity

A kit is commercially available to perform rapid tests to determine the antibody titer in serum against FPV, FCV and FHV-1 (Day, Horzinek, &

Schultz, 2015). This test has been validated and applied in several studies (DiGangi, Gray, Levy, & et al., 2011).

In the case of FPV, there is an excellent correlation between the presence of protective antibodies and resistance to infection (Lappin M. , 2012), so it is possible to determine the presence of antibodies using this kit which has a specificity of 89% and a sensitivity of 79% (Mende, Stuetzer, & Truyen, 2014), or a specificity of 99% and a sensitivity of 49% (DiGangi, Gray, Levy, & et al., 2011) when compared with a hemagglutination inhibition test. If the result is negative, meaning the cat has a low or no antibody titer, revaccination is recommended. On the contrary, if the result is positive, revaccination is not required (Day, Horzinek, & Schultz, 2015).

For FCV and FHV-1, the correlation between protective antibodies and resistance to infection is much less robust, mucosal and cell-mediated immunity prevail. For this reason, the use of this kit is not reliable to determine whether or not revaccination against FCV and FHV-1 is required (Lappin, 2012).

In the case of FPV, these tests can be used to verify a successful response to vaccination in puppies, determine whether there is protection in adults and then decide whether or not to revaccinate and for control in case of FPV outbreaks in kittens (Day, Horzinek, & Schultz, 2015).

There is also an antibody test for FIV, but it should be noted that it is used for diagnostic and not for prevention and has no value to determine the protective immunity against the disease. If FIV infection is suspected in regularly vaccinated cats, it is preferable to use a discriminating serological test or validated PCR test (Day, Horzinek, & Schultz, 2015)

2.3.2 Poor responder animals

The body is not always able to establish an adequate immune response following vaccination. If the vaccination procedure was done correctly, the reasons why the animal does not respond correctly to a vaccine could be the following: interference from MDAs (this is the most common cause), the animal is immunosuppressed as a result of stress or ongoing disease, genetic factors in the animal prevent recognition of vaccine antigens. Some parasites and viruses can induce immunosuppression. No animal with severe disease or ongoing febrile states should be vaccinated. Stress reduces the immune response as a result of high steroid production; examples of stressful situations are pregnancy or a state of malnutrition or altered body temperature such as hypo and hyperthermia. Even in these cases, the animal should not be vaccinated unless it is strictly necessary.

Genetic factors that can compromise a proper immune response concern certain breed of dogs; there are no data in the literature confirming a similar dynamic in cats. All these categories can be grouped as "non-responders", i.e. individuals in whom vaccination does not trigger the establishment of an immune response. These individuals are present in much smaller numbers than those who typically respond to a proper vaccination, so in a random population of animals, the range of immune responses tends to follow a normal distribution: there are excellent, average and poor responses.[2]

[2] This topic refers to: Tizard Ian, *Veterinary Immunology* 9ted., Saunders, 2013

3. ADVERSE VACCINAL EVENTS IN FELINE

Even though vaccines have been used for many years and the principles of vaccination are well known, the practice of vaccination is continuously evolving, trying to improve more and more efficacy and safety. [3]

The first vaccines used, besides having a limited efficacy, produced significant side effects, even though in terms of risk/benefit ratio, these adverse effects were mainly considered acceptable if the alternative was to contract a potentially lethal infectious disease. Continued developments in vaccinology have allowed for a reassessment of risk versus benefit, thus leading to a change in protocols.

"Vaccination is not always a harmless practice. For this reason, the use of any vaccine should be accompanied by a risk-benefit analysis conducted by the veterinarian in consultation with the animal owner. Vaccination protocols should be tailored to each animal, giving due consideration to the severity of the disease, the zoonotic potential of the agent, the animal's risk of exposure, and any legal requirements related to vaccination." (Tizard, 2013). Safety and efficacy are the two key factors that determine vaccine use. The risks of vaccination should not exceed the risks associated with the possibility of contracting the disease. For example, it may not be appropriate to use a vaccine for a rare disease, easily treated with another type of therapy, or is of little clinical importance. In addition, the unnecessary use of a vaccine may interfere with a very common diagnostic practice such as serum antibody testing. In conclusion, with regard to the safety factor of a vaccine, its use must be related to the degree of risk of disease and

[3] This topic refers to: Tizard, I. *Veterinary Immunology* 9th ed., Saunders, 2013

the availability of alternative and specific control procedures or treatments in terms of efficacy and safety.

Regarding the efficacy of a vaccine, it must be kept in mind that a vaccine is not always effective. "In some diseases, such as equine infectious anemia, Auletian disease in mink, and African swine fever, little or no protective immunity can be induced even with the best vaccines. In other diseases such as foot-and-mouth disease in pigs, the immune response is transient and relatively ineffective, and effective vaccination is difficult to achieve." (Tizard, 2013).

Consequently, animal vaccines are categorized according to their importance, as I have described in previous chapters. Vaccines that are considered essential, because they protect against common and dangerous diseases, fall into the first category (core vaccines), and failure to use them would expose the animal to a high risk of disease or death. The choice of decreeing a vaccine essential or not varies according to geographical location, where a disease may or may not be endemic. To the second category belong the non-essential vaccines; therefore optional (or non-core), directed towards diseases whose risk associated with a lack of vaccination is low.

The risks associated with these diseases are very often determined by the location and lifestyle of the animal. Therefore, the veterinarian must determine the use of non-core vaccines must be determined by the veterinarian based on a risk/benefit analysis based on the lifestyle of the animal and its exposure to the risk of contracting the disease.

The third category represents that of non-recommended vaccines, vaccines whose risks significantly outweigh the benefits. It is important to remember that the veterinarian's use of any vaccine must be conducted based on informed consent. Thus, the owner must be made

aware of the risks and benefits related to the recommended vaccination.

Three principles should be applied to determine whether a vaccine causes an adverse effect (Tizard, 2013):

I. Is the effect consistent? Clinical responses should always be the same if the vaccine is administered to a different group of animals, by different investigators, and regardless of the method of investigation.

II. Is the effect specific? The event should be explicitly related to the vaccine in question. For example, an adverse event can be caused by adjuvants and additives and not necessarily by the active component.

III. Is there a temporal relationship? The first manifestation of the adverse reaction must necessarily follow the administration of the vaccine and not precede it.

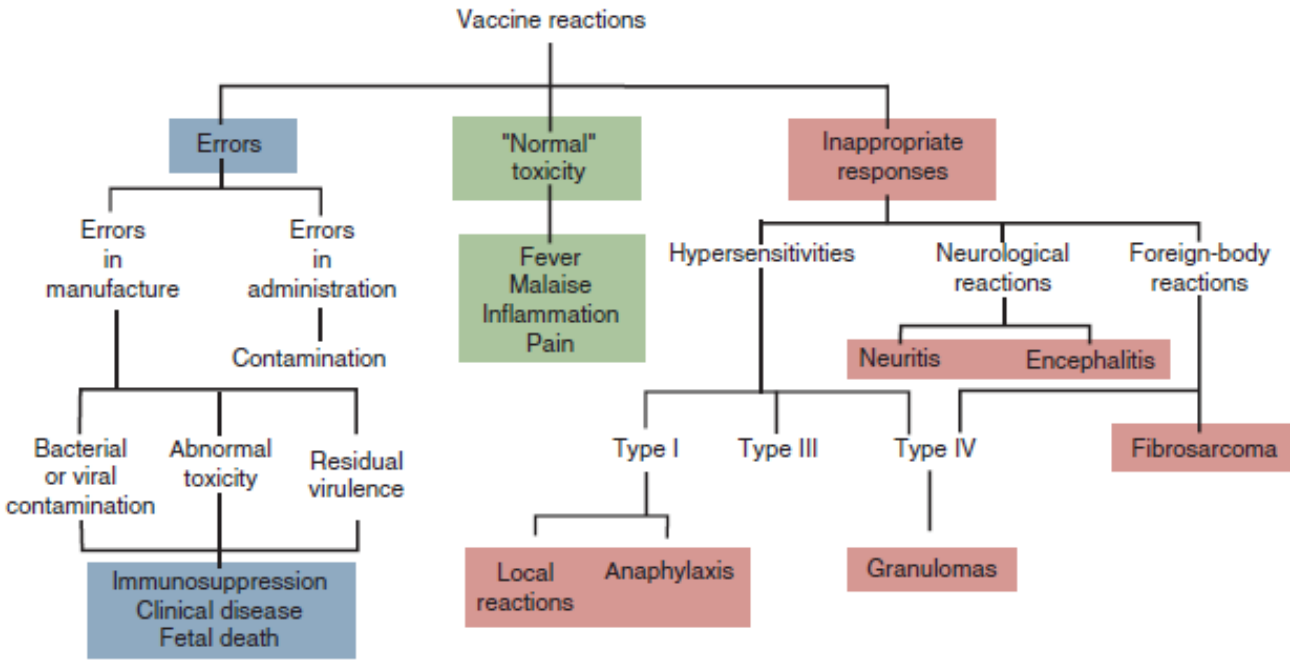

Figure 4 A simple classification of the major adverse effects of vaccination. (Tizard, 2013)

3.1 Failure of vaccination

There are many reasons why a vaccine may not confer protective immunity on an animal.

- Improper administration: in most cases, the lack of efficacy of a vaccine is due to incorrect or unsatisfactory administration. For example, poor storage may be the cause of the inactivation of a live vaccine. The use of antibiotics in combination with live bacterial vaccines, chemicals to sterilize the syringe, or excessive use of alcohol to dab the skin can interfere with the action of a vaccine (Tizard, 2013).
- Vaccine administration[4]: almost all vaccines are administered by injection, with care and consideration for the animal's anatomy. A wrong method of administration can injure or introduce infections in the animal (Tizard, 2013).

  Therefore it is necessary to use clean and sharp needles, which would otherwise cause tissue damage and infections at the injection site. Moreover, the animal's skin must be clean and dry without exceeding the use of alcohol. Vaccines are given in standard doses, and therefore a dose cannot be divided according to the animal's weight; a vaccination must not take into account the size of the animal nor its age. There is enough antigen in each dose to trigger an immune response regardless of the animal's weight. The most common method of administration is a subcutaneous or intramuscular injection; this approach is suitable for single-animal vaccinations (and not mass vaccinations, as is the case in animal husbandry) and for diseases to which a systemic immune response is essential. In

[4] This topic refers to: Tizard Ian *Veterinary Immunology* 9th ed, Saunders, 2013, p. 313-323

some diseases, systemic immunity is not as important as local immunity; in these cases, it would be more appropriate to administer vaccines at the site of a potential invasion. For example, intranasal vaccines are available for feline infectious rhinotracheitis, *Bordetella bronchiseptica* infections, coronavirus, and feline calicivirus. "Intranasal vaccination stimulates good local secretory IgA production and local cell-mediated, nonspecific immunity (e.g., type I interferons)" (Day, Horzinek, & Schultz, 2015). "Great care must be taken to administer the product by the route for which it is registered. If MLV vaccines containing parenteral (i.e., subcutaneous) FCV and FHV-1 are used locally (i.e., intranasally or orally), they can cause serious problems in the cat" (Day, Horzinek, & Schultz, 2015).

It has become common use, for convenience, to use mixtures of antigens within a single dose of vaccine; for example, in the cat are registered in Italy polyvalent vaccines that contain the antigens of FCV, FPV, FHV-1 and FeLV. However, it may be wasteful to use vaccines against organisms that may not cause problems.

The concern that the use of mixtures of multiple antigens may lead to less adequate protection (competition between antigens may occur, but vaccine manufacturers modify antigen doses to take that into account) or an increase in side risks is unfounded; there is no evidence to support the claim that the risk of adverse effects increases disproportionately with mixtures of multiple antigens (Tizard, 2013).

- Non-response: it may happen in some cases that the vaccine turns out to be ineffective (Tizard, 2013).

The production method may have destroyed protective epitopes, or an antigen may be insufficient in the vaccine. Cases like these are rare; more commonly, an animal may fail to establish an immune response. The immune response is a biological process, and as such, it is not absolute; protection may not be enough and is never the same for everyone in a vaccinated population. Most animals respond to antigens by mounting an average immune response; some excellent, and a small portion will have an inadequate immune response. It is essentially impossible to confer 100% protection on a random population of animals with vaccination (Tizard, 2013). The size of the unresponsive portion of the population will vary from vaccine to vaccine, and its importance will be relative to the nature of the disease. If the disease is of a highly contagious type, the presence of unprotected animals may still allow the disease to spread, as in the case of foot-and-mouth disease. In contrast, 70% protection may be sufficient to stop disease transmission in a population effectively for diseases such as rabies (Tizard, 2013).

Another type of failure occurs when the normal immune response is suppressed. Severe parasitosis or nutritional deficiencies lead to the animal being immunosuppressed; vaccination is not recommended in these cases. Likewise, animals with high fever or severe illness should not be vaccinated. Another factor that can reduce the immune response is stress, the cause being increased steroid production. Examples of stress include pregnancy, fatigue, malnutrition, hypo and hyperthermia.

As discussed in previous chapters, the primary cause of failure to elicit an adequate immune response in puppies is interference

between vaccine action and MDAs. Recent studies of 10,483 dogs of varying age, breed, and size examined factors influencing seroconversion after rabies vaccination (Tizard, 2013). Differences in antibody titers were found related to size (small dogs produced higher numbers of antibody titers than large dogs), breed (significant failure rates in Labradors and German Shepherds), and age (juveniles less than one year of age produced a lower antibody response than adults where peak antibody response was found at ages 3-4 years). What did not have a detectable influence on failure rates was gender.

Failure rates also varied widely among vaccines: from 0.2% in the worst case to 0.01% in the best, and some vaccines showed a significant difference in efficacy from batch to batch. The analysis results reported that 19% of the variation in antibody titers was due to differences between vaccines, 8% due to differences in breed, 5% due to size, and 3% other. Similar variables likely influence the responses of dogs and cats; consideration should be given to reformulating vaccines based on these variables (Tizard, 2013).

- Failure in correct administration: even with proper administration, adequate dose, and effective vaccine, some animals may not be protected.If the vaccinated animal was incubating the disease before vaccination, then the vaccine will not have protective efficacy as it will be too late to affect the course of the disease. Alternatively, a failure to protect may be caused by a vaccine containing the wrong strain or antigens, thus not protective against the disease (Tizard, 2013).
- Manufacturing or administration errors: problems associated with the use of vaccines may be due to incorrect manufacturing or

administration. Some MLV vaccines against FCV and FHV-1, when administered intranasally, may retain the ability to cause disease, spread to the oropharynx, and cause persistent infection. In addition, these vaccines can infect other animals that have come in contact with the vaccines. MLV vaccines against parvovirus can cause a transient decrease in lymphocyte responses, lymphopenia, and immunosuppression. Typically, immunosuppression is seen between 5 and 11 days after vaccination. Vaccination against FHV-1 can reactivate latent infections (Tizard, 2013).

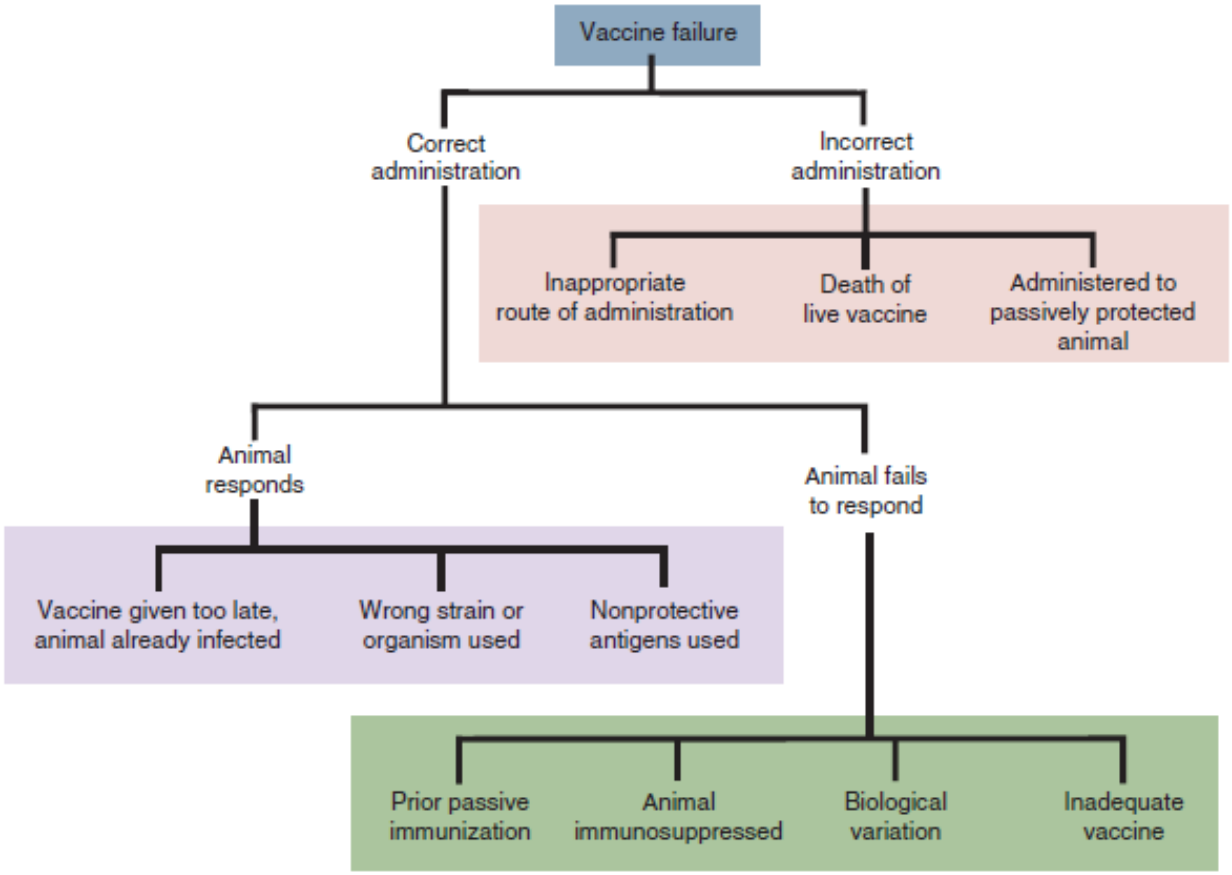

Figure 5 A simple classification of the ways in which a vaccine may fail to protect an animal. (Tizard, 2013)

3.2 “Normal” toxicity

A mild inflammatory response is necessary to induce an efficient immune response that is protective, so it is common for vaccines to cause momentary inflammatory reactions. This may cause pain at the site of inoculation. In addition, local, firm or edematous swelling may develop that is warm to the touch. These reactions appear about one day after vaccination and may last about a week. Generally, these swellings disappear without consequence unless an abscess develops. Bacterial vaccines that contain inactivated Gram-negatives can be toxic because endotoxins are inoculated that cause the release of cytokines leading to fever, leukopenia, and shock. Such reactions are sufficient in the pregnant female to cause an abortion (Tizard, 2013).

3.3 Hypersensitivity reactions

Vaccines can cause rare but severe allergic reactions. Type I hypersensitivity is an immediate response to an antigen that occurs within minutes to hours after exposure to an antigen. It occurs when an animal produces IgE in response to the immunizing antigen and other antigens in the vaccine, such as egg or tissue culture cell antigens. All hypersensitivity reactions are commonly associated with simultaneous inoculation of multiple antigens therefore associated with inactivated and adjuvanted vaccines. The short time frame in which this type of hypersensitivity occurs leads to its exclusion from anything occurring beyond 3 hours post-vaccination. Type III hypersensitivity reactions are equally dangerous: symptoms range from intense local inflammation to generalized vascular disturbances such as hemorrhagic purpura. In addition, vaccines such as rabies can induce a local complement-mediated vasculitis leading to ischemic dermatitis and local alopecia. Type IV hypersensitivity reactions are recognized by the presence of granulomas at the site of inoculation. Type IV is commonly associated with vaccines adjuvated with alum, oil, or aluminum hydroxide. These may be initially sterile granulomas or abscesses that become infected if the skin is soiled at the site of inoculation (Tizard, 2013).

3.3.1 Type I

Hypersensitivity reactions of type I[5] are a form of acute inflammation that occurs when mast cells have IgE on their membrane, which comes into contact with the antigen (or allergen) trigger an uncontrolled release of granules present inside mast cells, such as

[5] This topic refers to: Tizard Ian *Veterinary Immunology* 9th ed, Saunders, 2013, p.372-390

histamine and other vasoactive molecules that increase vascular permeability.

The uncontrolled release of these granules causes acute inflammation. Mast cells present IgE on the membrane following initial exposure to the allergen. This process is called the "sensitization" of mast cells to the antigen. IgE develops quickly, about a few seconds or minutes after exposure to the antigen.

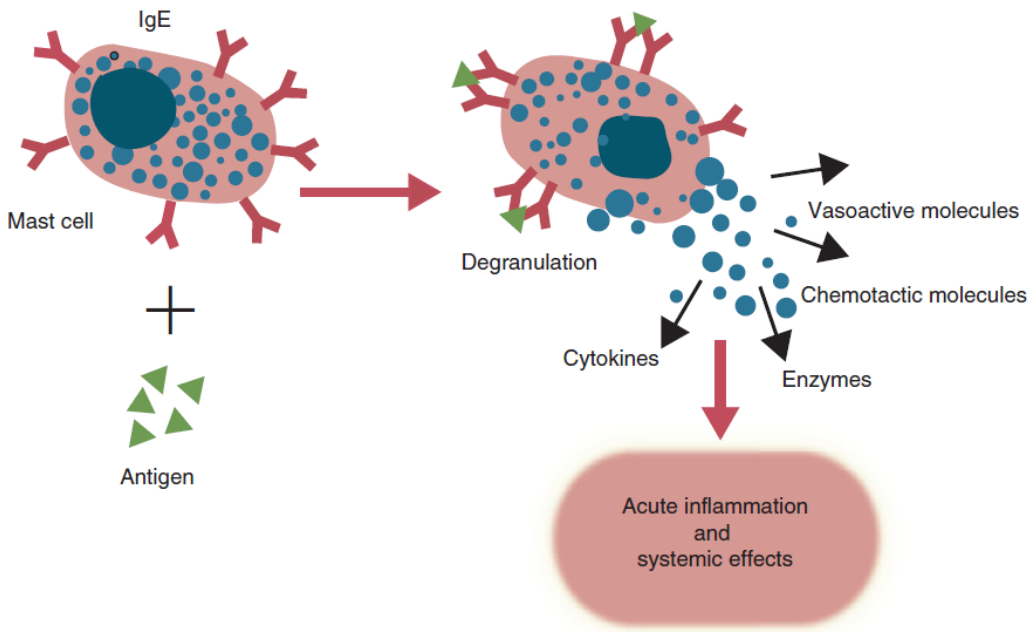

Figure 6 The mechanism of type I hypersensitivity reactions. (Tizard, 2013)

This type of hypersensitivity is called allergy, and the antigens that cause this inflammation are called allergens. If a type I hypersensitivity reaction is systemic, it is called allergic anaphylaxis or anaphylactic shock, which is life-threatening. A reaction similar to allergic anaphylaxis but not immune-mediated is called an anaphylactoid reaction.

The clinical signs of type I hypersensitivity result from a sudden and excessive release of inflammatory cells such as mast cells, basophils, and eosinophils. The severity and location of these reactions depend

on the animal's sensitization to the antigen, the amount of the antigen itself, and the route of administration. Allergic anaphylaxis occurs when the body cannot adapt the vascular system to changes induced by the release of vasoactive molecules by mast cells.

A type I hypersensitivity response can result from the administration of an antigen, including vaccines. In big and small animals, administration of a viral vaccine, particularly if adjuvanted, can elicit an IgE response to proteins present in the vaccine. These are proteins present on the cell culture used to develop the virus; if the virus is cultured on animal cells, the most common antigens are bovine serum proteins.

Another antigen targeted for a hypersensitivity reaction are proteins released from the cells used to grow the virus. When the virus is grown in eggs, some egg proteins may be target antigens, or a stabilizer such as gelatin may trigger an innate immune response.

Hardly the virus itself is the cause of a type I hypersensitivity reaction and thus the cause of a mistaken immune response. Therefore, an exaggerated reaction is more likely to occur in vaccines that contain traces of fetal calf serum, gelatin, or casein.

The vaccine manufacturing process varies with the manufacturer and the type of adjuvant used, but in general, viral antigens cannot be completely purified, so tissue culture products are entirely removed from the final product. For most patients, this is not a problem. However, in the population of patients with atopy (those who readily produce IgE responses and are often allergic), the stimulation of an IgE response by these non-target antigens presents a potential problem.

The non-target antigens in multiple viral vaccines mean that whenever a patient receives a vaccine containing the non-target antigens, those same non-target antigens are available to re-stimulate the immune response. Severe allergies have been associated with the use of rabies vaccines.

Symptoms of an anaphylactic reaction vary from species to species: in cats, the main organ involved in a shock reaction in the lung. Cats undergoing allergic anaphylaxis show vigorous scratching around the face and head because histamine is released into the skin. This is followed by dyspnea, salivation, vomiting, incoordination, collapse, and death. Necropsy reveals bronchoconstriction, emphysema, pulmonary hemorrhage, and edema of the glottis. The major mediators in the cat are histamine and leukotrienes.

Treatment of type I reactions should be symptomatic, depending on the severity and type of symptoms. Indicated medications (used alone or often in combination) include:

1. H1 antihistamines to block histamine receptors in the immediate phase.

2. Fast-acting glucocorticoids to block arachidonic acid products during the late phase or in the state of anaphylactic shock.

3. Adrenaline

4. Intravenous crystalloid fluids to compensate for the hypovolemic shock state.

In case of respiratory distress and cyanosis, oxygen therapy should be associated.

3.3.2 Type III

"Immune complexes formed by combining antibodies with antigen activate the classical complement pathway. When these complexes are deposited in tissues, activated complement generates chemotactic peptides that attract neutrophils. The accumulated neutrophils can then release oxidants and enzymes, causing acute inflammation and tissue destruction. Lesions generated in this manner are classified as type III or hypersensitivity mediated complex immune reactions." (Tizard, 2013).

The Arthus reaction is a typical type III hypersensitivity response[6], occurs within 24 hours of vaccine administration, and is localized at the inoculation site.

Clinical signs are swelling and pain at the site of inoculation. Histologically, the Arthus reaction is classified as vasculitis (inflammation of blood vessels) with neutrophil infiltration.

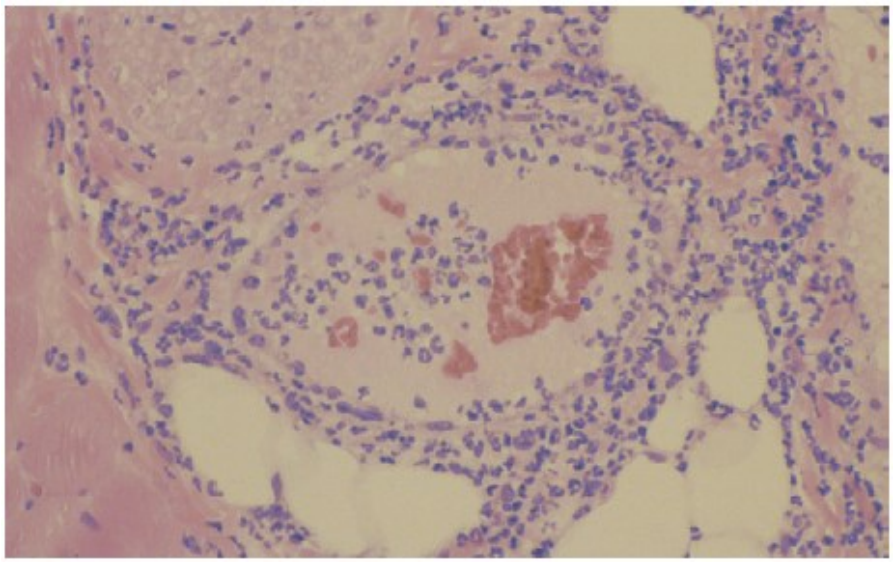

Figure 7 A histological section of an Arthus reaction in the skin of a cat. (Courtesy Dr. A .Kier.), (Tizard, 2013).

6 This topic refers to: Tizard Ian *Veterinary Immunology* 9th ed., Saunders,2013, p.403-411

These reactions occur when a large amount of IgG is circulating in the body (because the subject has been previously sensitized), specific for target or non-target antigens. When an antigen is injected into the tissue (for example, with the administration of a vaccine), immunocomplexes are formed (antigen bound to antibody) deposited mainly at the level of blood vessels in the dermis, causing inflammation. Complement fixation causes chemotactic factors, C3a and C5a, which cause mast cell degranulation and neutrophil infiltration. The inflammation causes swelling and pain in the area.

Symptoms generally resolve after 2 to 3 days. Therefore, determining an antibody titer against the target antigen is a logical step to take when deciding when and whether to revaccinate the patient.

3.3.3 Type IV

Delayed hypersensitivity: takes longer than 12 hours to develop and involves a cell-mediated immune response rather than an antibody response to antigens. Delayed hypersensitivity thus indicates the presence of antigen-specific CD4+ T cells. After activation, these T cells release pro-inflammatory cytokines, such as interferon-ɣ, TNF, IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF), which attract and activate macrophages. GM-CSF is a glycoprotein secreted by T cells, natural killer cells, and fibroblasts that functions as a cytokine. Chronic T-cell stimulation and cytokine release can lead to granulomas composed of macrophages and lymphocytes (Moore & HogenEsch, 2010).

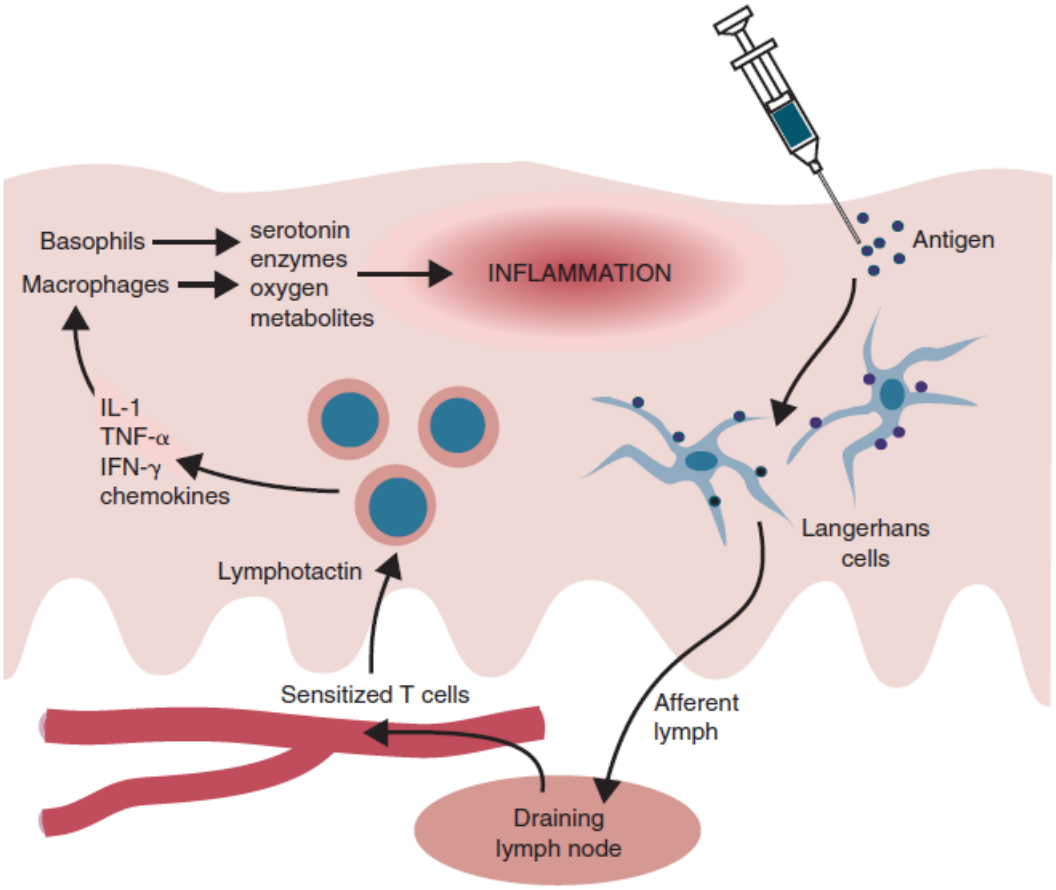

Figure 8 A schematic diagram depicting the mechanism of a delayed hypersensitivity reaction. (Tizard, 2013)

3.4 Feline injection-site sarcoma

Feline Injection-Site Sarcoma[7] (FISS) is a malignant tumor of connective tissue or mesenchymal origin that has been known since the early 1990s. The first description of FISS was reported in the Journal of the Veterinary Medicine Association in 1991 in an article written by Hendrick and Goldschmidt (1991) entitled "Do injection site reactions induce fibrosarcoma in cats?".

There was an initial correlation with rabies vaccination and vaccination against feline leukemia virus, subsequent studies have shown that the onset of this malignant neoplasm occurs as a result of an abnormal reaction of tissues to chronic inflammation induced by the inoculation of a substance, even though the substance inoculated may be a vaccine or other.

An increased incidence of FISS observed in the United States associated with rabies vaccination led to the coining of the term "vaccine-associated sarcomas".

Now more properly called "injection-site sarcoma" because recent studies on the pathogenesis of this neoplasm have shown that in addition to vaccines, other material such as antibiotics or steroids (Kass, et al., 2003), the pesticide lufenuron (a benzole derivative of urea) (Esplin & McGill, 1999), non-absorbable suture material (Buracco, Martano, Morello, & Ratto, 2002) and microchip implants (Daly, et al., 2008) can lead to the onset of this condition. In 1996 a task force (Vaccine-Associated Feline Sarcoma Task-Force) was established to widen the knowledge of the epidemiology, etiology, and treatment of FISS as much as possible. This work ended in 2005 with

[7] This topic refers to: Martano M., Morello E., Buracco P. Feline injection-site sarcoma: past,present and future perspectives. Vet J. 2011 May; 188(2):136-141.

a document describing the progress achieved on the knowledge of the topic (VAFSTF, 2005).

A retrospective epidemiologic study was published by Kass et al. (1993) of 345 cats with a diagnosis of fibrosarcoma, vaccination history, and known site of tumor development. At the end of the study, it was observed that the incidence of the disease was higher than two peaks of the age of 6-7 years and 10-11 years.

A relationship between the onset of FISS and vaccination for FeLV and rabies was observed. The risk was directly proportional to the number of injections: it was 50% wider after a single administration and subsequently increased by 127% and 175% more after two and three administrations of the vaccines mentioned above, respectively.

The histologic finding of grey-brown material in the necrotic centre and cytoplasm of macrophages (Hendrick & Dunagan, 1991) was consistent with an inflammatory-type reaction elicited by inoculation of foreign material potentially caused by vaccination. However, epidemiologic data on FISS are inconsistent, ranging from figures of 1/1000 and 1/10,000 vaccinated cats (Lester, Clemett, & Burt, 1996) to 0.63/10,000 (Gobar & Kass, 2002)

This inconsistency in epidemiologic data is likely because adverse events resulting from vaccine administration are voluntarily reported by veterinary physicians directly to manufacturers or government agencies. Voluntary reporting results in underreporting of adverse events. In addition, there is no hard data on the total number of animals vaccinated (Tizard, 2013).

Histologically, these are mesenchymal tumors of different types: fibrosarcoma is the most frequently observed type, but cases of

malignant fibrous histiocytoma, osteosarcoma, chondrosarcoma, rhabdomyosarcoma, and undifferentiated sarcoma have also been documented. These types of neoplasms appear to originate from a proliferation of myofibroblasts and fibroblasts located at the site of chronic inflammation, abnormal tissue response to the inoculation of a substance foreign to the body (Hendrick & Brooks,1994).

In addition to the proliferation of these cells, mutation of oncosuppressor genes such as the p53 gene appears to contribute to the etiopathogenesis (Hendrick M. , 1998) Other factors such as the use of vaccines at colder than storage temperatures appear to be associated with an increased risk of FISS occurrence (Macy, 1999) (Kass, et al., 2003). This multifactorial etiology could explain the low incidence of FISS.

The diagnosis of FISS begins with the report of a rapidly growing mass developed on common injection sites.

Latency time varies widely: from 3 months post-injection to 10 years (McEntee & Page, 2001) (Seguin, 2002); this is another variable contributing to the difficulty in determining incidence. Regardless of the latency time, once the neoplastic proliferation process is triggered, the mass can grow several centimetres in diameter within weeks.

Therefore, the VAFSTF recommends biopsy of the mass according to the "3-2-1 rule": persistence (beyond three months post-injection), size of the mass (greater than 2 cm), and rapidity of size increase (after one-month post-injection).

Histologic specimens of FISS are characterized by infiltrates of inflammatory cells such as lymphocytes and macrophages, granulation tissue, and multinucleated neoplastic cells. The histology of FISS was

first described by Doddy et al. (1996) on analysis of 165 tissue samples. Amorphous material of grey-brown coloration present in the central areas of multinucleated neoplastic cells could represent remnants of the vaccine itself or aluminum salts used as an adjuvant, according to authors Hendrick and Dunagan (1991). The finding of cells with features indicating malignancy (such as central necrosis, a high number of mitotic figures, and cellular pleomorphism) is more common in these injection-related tumors than in sarcomas of another origin.

The infiltrative nature of FISS makes it challenging to plan an intervention; imaging methods such as abdominal ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI) can help verify the extent of the neoplasm.

An effective cure for FISS has not yet been found, but a multimodal approach can bring positive results. The most crucial therapy point is surgical excision of the primary tumor, including wide margins of 3-5 cm of healthy tissue. Other procedures such as amputation of the spinous vertebral process, scapulectomy, or limb amputation have been necessary in more severe cases. FISS has a recurrence rate as high as 45%, even after surgery (Cronin, et al., 1998).

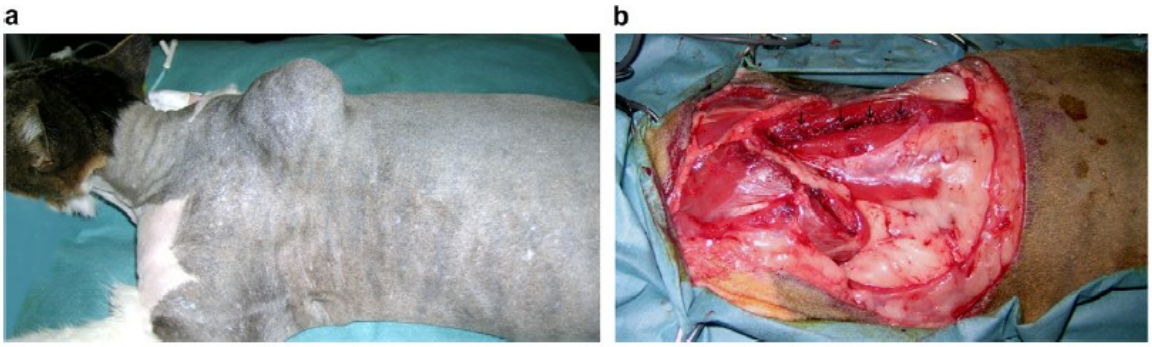

Figure 9 Resection of an interscapular FISS. (a) Pre-operative view. (b) Intraoperative view. (Martano et al. 2011)

Combining radiation therapy and surgery with histologically clean margins may improve the disease-free interval (DFI). However, in a study described by Kobayashi et al., (2002), the tumor recurred in 42% of cases even with histologically clean margins. This makes the pathogenesis of FISS unique and calls into question the interpretation of margins in veterinary histopathology. The metastatic potential of FISS is low. Chemotherapy can be supportive; commonly used drugs are doxorubicin and cisplatin, but studies reported in the literature have not shown improvement in recurrence rates.

In conclusion, according to the VAFSTF, a multimodal approach including surgery, radiotherapy and chemotherapy is recommended. In addition, the VAFSTF has developed guidelines for veterinarians to understand better which substances are implicated in FISS formation.

The guidelines call for the administration of the FeLV vaccine in the left hind limb as distally as possible and administration of the rabies vaccine in the right hind limb, again as distally as possible. In comparison, the polyvaccine containing core vaccines should be administered in the right shoulder (Tizard, 2013).

A survey of 392 cats with FISS treated before and after December 31, 1996 (the year of the VAFSTF founding) reported a change in the areas of tumor development during that period. After 1996, the number of FISSs detected in the body cranially at the diaphragm had steadily decreased until an approximately equal distribution of neoplasms in the body in 2006.

According to VAFSTF guidelines, rabies vaccination was found to be the cause of 51.7% of new cases, 28.6% FeLV, and a polyvalent

vaccine was found to be responsible for 19.7%. This study revealed the effective use of guidelines by veterinary physicians and confirmed the perception of FISS as a problem. It also confirmed that injections are the cause of the disease.

In conclusion, the VAFSTF recommends not vaccinating more than necessary and alternating inoculation sites, giving injections as far from the spine as possible (not just vaccines but any injection).

The authors of the article from which I took the notions for this paragraph wondered if the incidence of FISS is decreased with the new guidelines of the VAFSTF of 2005, it would be interesting to evaluate it.

3.5 Adverse effects of adjuvants

An immune response to an external pathogen can cause tissue damage during eliminating the pathogen and result in some clinical signs of disease. Adjuvants as agents that can amplify the potency of the immune response can likewise amplify adverse effects. These adverse effects are related to antigen-adjuvant binding and are usually systemic and nonspecific, such as fever, lethargy, arthritis, anorexia, and soreness. They can also increase the likelihood of an autoimmune reaction; for example, excessive doses of IL-2 (cytokine) used as an adjuvant have been linked to autoimmune disease. (Vogel, 2000) (Meyer, 2001) (Hughes, 1998) (Kersten & Crommelin, 1995). Autoantibodies have been detected after the administration of the rabies vaccine and parvovirus (Hughes, 1998).

Adverse effects may also be given by the chemical nature of the adjuvant itself. For example, crude saponins can cause hemolysis when injected intravenously (Kersten & Crommelin, 1995). More frequently, adjuvants are responsible for local reactions, including inflammation and more rarely granulomas or sterile abscesses. In cats, the vaccine most often correlated with non-neoplastic reactions is the rabies vaccine (Meyer, 2001). Adjuvants with a depot effect can cause granuloma formation that resolves after a few weeks. This issue primarily affects farm animals more than companion animals (Meyer, 2001) (Morrison & Start, 2001).

At last, after vaccination, local inflammation and granulomas have been linked to the development of injection-induced sarcomas in cats. Some hypotheses attribute a role in the pathogenesis of these neoplasms to adjuvants containing residual aluminum that causes an abnormal inflammatory reaction (Hendrick M. , 1998).

Vaccines primarily related to this neoplasm are rabies and feline leukemia vaccines. (Hendrick M. , 1998) (Kass, Barnes, Spangler, et al., 1993).

The exact role of antigens, adjuvants, or other factors in sarcoma development remains determined, but circumstantial evidence suggests that adjuvants may be involved, and the timing of sarcoma development is suspect. Sarcomas have become more familiar with the increasing use of adjuvanted vaccines.

In the 1980s, the first FeLV vaccines were produced and brought to market and modified live rabies vaccines were replaced by switched-off adjuvanted vaccines (Morrison & Start, 2001).

Adjuvants also increase the inflammatory response, which appears to be an important risk factor for these sarcomas (Morrison & Start, 2001) (Doddy, Glickman, Glickman, & Janovitz, 1996) (Macy, 1999).

Apparently, some vaccines cause inflammatory granulomas, which can, in some cats, develop into a malignant neoplasm. Aluminum adjuvants can cause inflammation, and some authors suggest that they should be avoided in cats (Couto & Macy, 1998). However, these considerations remain controversial because the pathogenesis of FISS has not yet been elucidated, largely remains unknown, and adjuvants other than aluminum and non-adjuvanted vaccines have also been linked to cases of FISS (Macy, 1999), (Hendrick M. , 1998) (Couto & Macy, 1998).

Generally, it can be difficult to determine the incidence of rare adverse effects for any particular vaccine. Although veterinary vaccines must be labelled with adverse effects observed during premarket testing, veterinary vaccine manufacturers are not required to update labels

with adverse effects observed after marketing or record reports from veterinarians (Meyer, 2001). There may also be a disincentive to routinely list postmarketing adverse effects because veterinarians may assume that vaccines with more adverse effects listed are more dangerous (Meyer, 2001). Although these potential hazards must be considered, killed vaccines are generally considered safer than modified live vaccines, which can induce disease in immunocompromised animals (Roth, 1999).

In most cases, the adverse effects of adjuvants are mild, and in general, their benefits outweigh the dangers of their use. In specific situations such as cat vaccinations, the benefit versus the danger may differ and need to be considered more carefully[8].

[8] This topic refers to: Spickler AR, Roth JA. Adjuvants in veterinary vaccines: modes of action and adverse effects. *J Vet Intern Med*. 2003 May.Jun; 17(3):273-281

CONCLUSION

Vaccination is the only reliable, safe and effective way to protect animals from infectious diseases.

Although vaccine-related toxicity is rare, mild, and temporary, the use of vaccines is not without risk. Before using a vaccine, the veterinarian must consider the probability of an adverse effect occurring, the possible consequences, and the severity of the adverse effect. All of this must be followed by an analysis of the risks versus the benefits given by the vaccination.

Potential risks include residual virulence, toxicity, allergic responses, possible disease onset in immunodeficient hosts, neurological complications, and fetal harm.

Adverse events are reported voluntarily by veterinarians to manufacturers or government agencies; the reported figures have been impossible to analyze satisfactorily for two reasons: reporting is voluntary, and there is very little data available on the number of animals actually vaccinated.

Voluntary reporting inevitably results in significant underreporting of adverse events, as they may be considered insignificant or inconvenient to report.

Vaccine manufacturers know the doses of vaccine sold but cannot calculate the number of animals vaccinated. In an outpatient study of 496,189 cats, the incidence of adverse events following administration of 1,258,712 doses of vaccine was examined (Tizard, 2013).

The researchers reported 2560 adverse events (51.6/10,000 cats vaccinated). The risk was most significant for cats under one year of age. For reasons still unknown, the risk was also higher in spayed cats than in unneutered cats. Lethargy was the most reported event. In addition, it was observed that the number of adverse events increased

significantly when multiple vaccines were administered during a single visit. However, it must be kept in mind that standard definitions of vaccine-associated adverse events are not available to date, and identification is based on the clinical judgment of the treating veterinarian and is therefore subject to distortion.

The technology behind vaccine production in continuous improvement, a greater understanding of immune mechanisms and ways to optimize the immune response to achieve maximum protection have allowed for more excellent safety and efficacy in the use of vaccines. These improvements allow for a reassessment of the risks and benefits of vaccination and have led to changes in guideline protocols. An evaluation of the pros and cons performed by the Veterinary Physician in consultation with the animal owner allows for the creation of vaccination protocols tailored to the animal's actual risk of exposure to an infectious disease, lifestyle, and age. The guidelines provide recommendations and protocols to control common infectious diseases, such as feline panleukopenia, rhinotracheitis, and calicivirus infections. They allow the veterinarian to choose a vaccination schedule that is appropriate to the animal's lifestyle, age and medical history. In the case of the cat, the veterinarian should take into account the studies carried out on the occurrence of injection-site sarcoma and then adapt the practice accordingly, taking precautions such as rotation of the site of inoculation of the vaccine and use more suitable inoculation sites such as the base of the tail or the distal part of a limb; in order to mitigate the severity of the consequences due to the possible occurrence of this neoplasm. Vaccination is a fundamental practice for controlling infectious diseases and safeguarding public and animal health. However, in no case, a vaccine should be administered more than necessary without taking into account the patient's lifestyle

and with the awareness that, as for any drug, even for the vaccine, adverse events may occur.

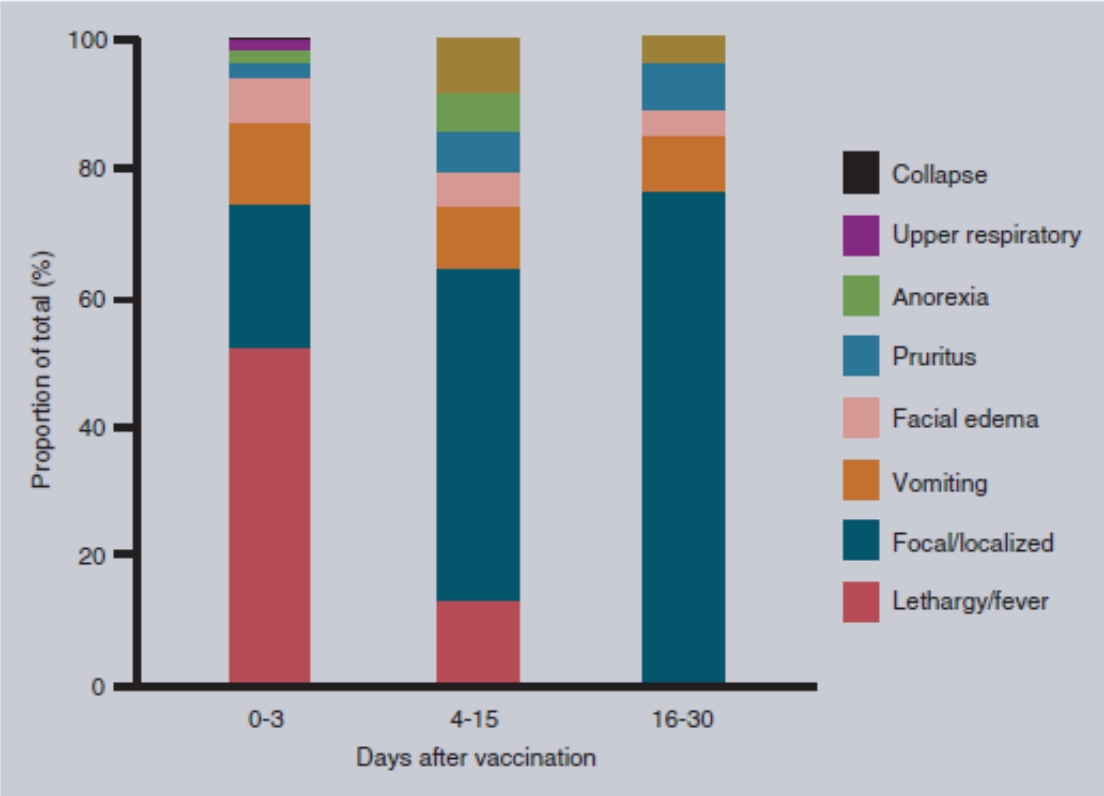

Figure 10 Distribution of types of vaccine-associated adverse events diagnosed during various periods after vaccination in 496,189 cats administered one or more vaccines from January 1, 2002 to December 31, 2004. (From Moore GE, DeSantis-Kerr AC, Guptill LF, et al: Adverse events after vaccine administration in cats: 2,560 cases (2002-2005), J Am Vet Med Assoc 231: 94–100, 2007.), **(Tizard,2013)**.

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