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Review

Production Technologies, Regulatory Parameters, and Quality Control of Vaccine Vectors for Veterinary Use

Raquel de M. Barbosa [1,2,*], Amélia M. Silva [3,4], Classius F. da Silva [5], Juliana C. Cardoso [6,7], Patricia Severino [6,7], Lyghia M. A. Meirelles [1], Arnobio A. da Silva-Junior [1], César Viseras [2], Joel Fonseca [8] and Eliana B. Souto [8,9,*]

1 Department of Pharmacy, Federal University of Rio Grande do Norte, R. Gen. Gustavo Cordeiro de Faria, S/N—Petrópolis, Natal 59012-570, Brazil
2 Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Granada, Campus of Cartuja s/n, 18071 Granada, Spain
3 Department of Biology and Environment, School of Life Sciences and Environment, University of Trás-os-Montes and Alto Douro (UTAD), 5001-801 Vila Real, Portugal
4 Centre for Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro UTAD, 5001-801 Vila Real, Portugal
5 Departamento de Ciências Exatas e da Terra, Universidade Federal de São Paulo, Rua Arthur Riedel, 275 Diadema, São Paulo 09972-270, Brazil
6 Laboratory of Nanotechnology and Nanomedicine (LNMED), Institute of Technology and Research (ITP), Av. Murilo Dantas, 300, Aracaju 49010-390, Brazil
7 Industrial Biotechnology Program, University of Tiradentes (UNIT), Av. Murilo Dantas 300, Aracaju 49032-490, Brazil
8 Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, No. 228, 4050-313 Porto, Portugal
9 REQUIMTE/UCIBIO, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, No. 228, 4050-313 Porto, Portugal
* Correspondence: rbarbosa@ugr.es (R.d.M.B.); ebsouto@ff.up.pt (E.B.S.)

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Citation: Barbosa, R.d.M.; Silva, A.M.; Silva, C.F.d.; Cardoso, J.C.; Severino, P.; Meirelles, L.M.A.; Silva-Junior, A.A.d.; Viseras, C.; Fonseca, J.; Souto, E.B. Production Technologies, Regulatory Parameters, and Quality Control of Vaccine Vectors for Veterinary Use. *Technologies* **2022**, *10*, 109. https://doi.org/10.3390/technologies10050109

Academic Editor: Manoj Gupta

Received: 19 August 2022
Accepted: 20 October 2022
Published: 21 October 2022

Abstract: This paper presents a comprehensive review of the main types of vaccines approaching production technology, regulatory parameters, and the quality control of vaccines. Bioinformatic tools and computational strategies have been used in the research and development of new pharmaceutical products, reducing the time between supposed pharmaceutical product candidates (R&D steps) and final products (to be marketed). In fact, in the reverse vaccinology field, in silico studies can be very useful in identifying possible vaccine targets from databases. In addition, in some cases (subunit or RNA/ DNA vaccines), the in silico approach permits: (I) the evaluation of protein immunogenicity through the prediction of epitopes, (II) the potential adverse effects of antigens through the projection of similarity to host proteins, (III) toxicity and (IV) allergenicity, contributing to obtaining safe, effective, stable, and economical vaccines for existing and emerging infectious pathogens. Additionally, the rapid growth of emerging infectious diseases in recent years should be considered a driving force for developing and implementing new vaccines and reassessing vaccine schedules in companion animals, food animals, and wildlife disease control. Comprehensive and well-planned vaccination schedules are effective strategies to prevent and treat infectious diseases.

Keywords: vaccines; veterinary application; bacteria; toxins; antigenic residues

1. Introduction

In the last century, the relationship between humans and pets has grown considerably in different societies, although it is not culturally universal. Only in the US do pet owners spend thousands of dollars a year to maintain care of their dog or cat. In addition, many works have shown how pets might play an essentially positive role in animal-assisted therapy in several conditions such as post-traumatic stress disorders or autism, for example [1].

In parallel with these benefits, pets can become harmful transmitters of various diseases such as brucellosis, roundworm, skin mites, *E. coli*, salmonella, giardia, ringworms, and cat-scratch fever [2,3]. No less important and also necessary is the vaccination of poultry, cattle, horses, sheep, goats and pigs. Vaccine use promotes animal health, safety for humans and financial protection for farmers. The animal vaccination process is fundamental, preventing and eradicating the spread of multiple diseases [4].

Vaccines are biological agents exploiting the humoral immune system's capacity and/or cell-mediated immunity safely to induce an immune response by inducing the production of immunological memory against a specific antigen derived from an infectious disease-causing pathogen [5,6].

Most of the vaccines currently available for animals have protein or polysaccharide antigens in their composition [7]. It is generally classified as liquid or lyophilized preparations of live (attenuated) or non-live (inactivated or killed) microorganisms [6,8]. In the last few years, viral vectors and RNA/DNA vaccines have contributed significantly to developing new immunizing products for animals use [8,9].

Bacterial vaccines and toxoids are produced from cell cultures in vitro, or in embryonated eggs using appropriate and validated methods. The cases described in this review do not apply to bacterial vaccines prepared from cell cultures or live animals. The bacterial strain employed may be genetically engineered and the identification, the antigenic power, and the purity of each bacterial culture used must be carefully controlled. Bacterial toxoids or anatoxins are prepared from toxins by reducing their toxicity to an undetectable level or by complete toxicity neutralization using physical or chemical methods; therefore, toxoids induce the production of neutralizing antibodies [10].

There are cases of bacterial toxins that are weakened until no toxicity exhibition but with enough strength to induce the formation of antibodies and specific disease immunity caused by the toxin. Toxins are derived from selected strains of specific microorganisms cultured in suitable media, or they may also be obtained by other appropriate methods (e.g., chemical synthesis) Toxins are derived from selected strains of specific microorganisms cultured in suitable media. They may also be obtained by other appropriate methods, for example, chemical synthesis. However, bacterial toxins should be weakened to be used as the bioactive compounds in vaccine products, and they present low or any toxicity as a fundamental requirement [11].

Toxoids can be purified by adsorption using adjuvants such as aluminum phosphate, aluminum hydroxide, calcium phosphate, and others. Bacterial toxoids may be in the form of a clear, transparent, or slightly opalescent liquid. Adsorbed toxoids are presented in the form of suspensions or emulsions, and some may be lyophilized. Unless otherwise indicated, provisions and requirements specified for bacterial vaccines also apply to vaccines based on bacterial toxoids and products containing a mixture of bacterial cells and toxoids [12]. Although alum-precipitated tetanus and diphtheria toxoids had been used for human immunization for many years, their use has declined considerably because of the variability in the production of alum precipitated toxoids.

Viral vaccines are prepared from viruses grown in suitable cell cultures, tissues, microorganisms, or embryonic eggs. If there is no other possibility, viral vaccines may also be produced in live animals. The used virus strains can be genetically engineered. Liquid or lyophilized preparations are composed of one or more virus or viral subunits or peptides. Live viral vaccines are prepared from viruses with attenuated virulence or low virulence for the native target species. Inactivated vaccines are subjected to a validated method of virus inactivation and can be purified and concentrated [13,14].

In this way, vaccines based on vectors are liquids or lyophilized preparations of one or more non-pathogenic or low pathogenic live microorganisms (bacteria or virus), in which one or more antigen-expressing genes, which elicit a protective immune response against other microorganisms, are inserted [15]. The preparation methods, which vary depending on the type of vaccine, should ensure the integrity and the immunogenic power of the antigen and the prevention of contamination by foreign agents. The origin of the animal

products used in the production of vaccines for veterinary use shall meet the regulatory requirements [16].

Substances from other sources must meet the requirements of Regulatory Agencies and should be prepared to prevent any contamination of the vaccine by living microorganisms or toxins. Cell cultures used in the preparation of vaccines for veterinary use should also satisfy the requirements. It may be necessary to demonstrate the effectiveness of the inactivation method against specific potential contaminants. The use of embryonic eggs from specific pathogens flocks is required for the production of the primary seed batch in every passage of a microorganism to the working seed batch [17]. If there is no alternative to the use of animals or animal tissues in the production of veterinary vaccines, these must be free from specific pathogens, and their nature will depend on the species of origin and the target vaccination species [18].

Vaccination is the main approach to achieve the best cost-effective relationship to prevent economic losses and to increase the quality of life of animals. Figure 1 shows several vaccine technologies available for animals. In veterinary medicine, many immunogens are still produced using conventional technologies, such as attenuated vaccines. However, with the development of biotechnological tools, these are being used in vaccine development. These "modern" vaccine technologies are not just used to control infectious diseases but also to increase their productivity and the control of ectoparasites.

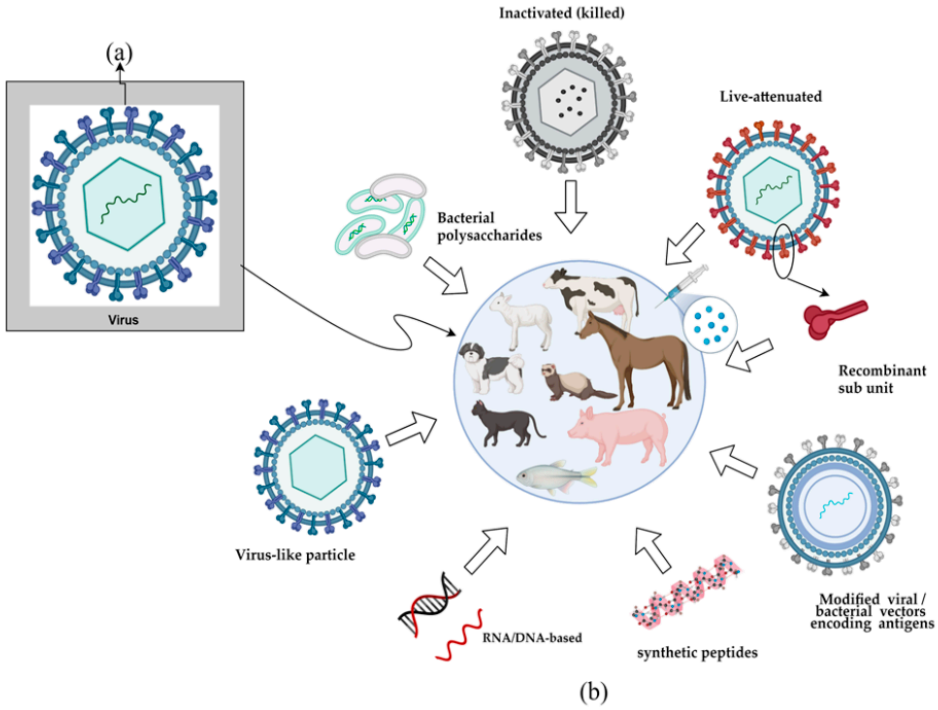

Figure 1. Schematic representation of common virus (**a**) and the main platforms adopted in the development of vaccines against pathogens (**b**).

There are currently several approaches to obtain a vaccine capable of promoting acquired immunity, from the most traditional ones, based on the intact pathogen (attenuated or inactive), or even based on the use of subunits, such as isolated proteins or self-assembled structural molecules, which are called virus-like particles, nucleic acids, or viral vectors. Table 1 shows the main characteristics of the mentioned vaccine platforms available for animals.

Among them, bacterial polysaccharide vaccines consist of inactivated or subunits that are characterized by structures that are part of the bacterial cell. It also constitutes purified molecules such as capsular polysaccharides, native or even recombinant proteins. With the advent of reverse vaccinology, several proteins identified the important targets in

bacterial infections being expressed in different vectors, purified, and tested as potential vaccine targets.

Another technology concerns the use of synthetic peptides, which are designed from studies by computational prediction, defining the possible sequences that contained immunogenic determinants [19]. The synthetic vaccine against *Rhipicephalus microplus* called SBm7462® was developed by the Laboratory of Biology and Control of Hematozoa and Vectors using this technique [20].

Table 1. Description of the most common types of vaccines for animal use.

Platform	Characteristics	Restrictions	Refs
Whole virus			
Attenuated	Entire virus passed on in successive cultivations to lose infective capacity.	Production requires cell culture of the virus and exhaustive safety tests as they are more immunogenic.	[21]
Inactivated	The intact virus is inactivated by chemical or physical methods.	Vaccines based on the inactivated virus require an initial high amount of virus.	
Subunit			
Proteins	Proteins or their fragments are injected directly into the animal.	Generally, require adjuvants or multiple doses to achieve the desired immune response.	[21]
Virus-like particle	Self-assembled viral structural proteins that resemble the virus, however, lack genetic material.	The biggest challenge of this platform is to ensure that the epitopes are in an adequate conformation after translation and that the expressed proteins are not allergenic.	[22]
Nucleic acid			
DNA	Insertion of the DNA that encodes the viral antigen into a plasmid.	They are platforms under experimentation for animal purposes.	[23]
RNA	Messenger RNA encapsulated in a lipid membrane.		
Vector encoding antigen			
Replicating and non-replicating	Non-infective pathogens are genetically modified with the insertion of one or more genes that express antigenic particles, which may or may not multiply in the animal organism.	Requires level 2 biosafety labs for production; it has reduced efficacy due to pre-existing immunity to selected vectors.	[24]

DNA vaccine is developed from a plasmid, and its expression contains genes encoding one or more immunogenic antigens of interest. Once these recombinant plasmids are inserted inside the host cell, the target gene will be transcribed. Recombinant RNA vaccines consist of fragments of the sequence of the genetic material of messenger RNA (mRNA), which can be designed to encode any viral, bacterial, or parasitic protein. When virus mRNA is inside host cells, they are translated into proteins, which induce an immune response to the host's body. In addition, customized RNA/DNA sequences allow researchers to create vaccines that produce virtually any protein desired [4,25–27].

Vaccines are essential to prevent and control zoonotic infectious diseases in humans and animals (domestic and wild). The use of vaccines in animals impacts positively the production and their quality of life. Some examples of veterinary vaccines are described in Tables 2 and 3.

Zoonoses have always been a great concern for the scientific community, with a strong worldwide public health impact, as recently happened with the COVID-19 pandemic. Data released by the WHO in 28 June of 2022 [28] confirmed 542,188,789 cases of COVID-19, including 6,329,275 deaths worldwide. In addition, the International Monetary Fund

predicted an estimated global cost to the economy of US$12.5 trillion by 2024 due to the new coronavirus pandemic. However, other zoonoses that also deserve mention are avian influenza and MERS, both with a high risk of becoming a new pandemic; and, as the regional transboundary epizootics, we can mention yellow fever, Venezuelan equine encephalitis, and Rift Valley fever [29].

Table 2. Examples veterinary vaccines using different strategies.

Vaccine Strategy	Disease	Animal	Consequences	Refs
Bacterial ghost construction	Avian Colibacillosis	Avian	Mortality of poultry bacterial infections—it causes a variety of disease manifestations in poultry including yolk sac infection, omphalitis, respiratory tract infection, swollen head syndrome, septicemia, polyserositis, coligranuloma, enteritis, cellulitis and salpingitis	[30,31]
Avirulent suspension of *Salmonella typhimurium* AWC 591	Salmonellosis	Commercial poultry	Economic losses and risks to public health such as diarrhea, fever, and stomach cramps	[32]
Modified live vaccine (MLV) infectious bovine rhinotracheitis	Rhinotracheitis	Cattle	Respiratory disease complex	[33]
The gene for protein 2 (VP2) of infectious bursal disease virus was cloned into a *Pichia pastoris* expression system	Infectious bursal disease (also known as Gumboro disease)	Avian	Immunosuppressive viral disease due to widespread destruction of lymphocytes	[34]
Replacement of the capsid-encoding gene (P1) from the vaccine strain O1 Manisa	Foot-and-mouth disease virus	Cattle, pigs, sheep, and many wildlife species	Economically devastating disease; reduced animal productivity and the restrictions on international trade in animal products	[35,36]
Recombinant vaccines based on *Brucella* Outer Membrane Protein (OMP) antigens	Brucellosis	Calves, sheep, cattle, goats, pigs, and dogs, among others	High economic losses due to restrictions on international trade in animal products; the signs and symptoms include fever, joint pain (arthritis, spondylitis, sacroiliitis), endocarditis and fatigue.	[37]
Recombinant vaccines based on their major toxins and their genetic origins (iota (ia), alpha (cpa), beta (cpb), and epsilon (etx), and toxoid vaccines, bacterin-toxoid vaccine	*Clostridial* diseases	Cattle, sheep, and goats	botulism, tetanus, enterotoxaemia, gas gangrene, necrotic enteritis, pseudomembranous colitis, blackleg, and black disease causing severe economic losses in livestock and poultry industries	[38]

Different zoonotic diseases are annually responsible for the death and economic loss due to the substantial reductions in livestock production. In general, the large-scale slaughter of herds negatively impacts the livestock sector, but this practice is essential to prevent human infections. In addition, wild animals can be mortally affected by other diseases such as West Nile disease (birds), yellow fever (neotropical monkeys), plague (black-footed ferrets), and Ebola (great apes) [29].

Table 3. Domestic animals' vaccination (cats, dogs, and rabbit) schedule examples. Recommendation from the National Office of Animal Health, representing the UK animal health industry.

Disease	Example (Supplier)/Vaccine Strategy	Recommended Vaccination Schedule
Feline Panleukopenia/ Infectious Enteritis (Parvovirus)	Fevaxyn® Pentofel (Zoetis Belgium SA)/Fevaxyn Pentofel contains the following inactivated viruses: feline panleukopenia virus, feline rhinotracheitis virus, feline calicivirus, feline leukemia virus, and the inactivated bacterium feline *Chlamydophila felis*.	Cats of 9 weeks or older. Two doses at an interval of 3 to 4 weeks.
Feline Calicivirus	Purevax RC (Boehringer Ingelheim, Ingelheim am Rhein, Germany)/Attenuated feline rhinotracheitis herpesvirus (FVH F2 strain) and inactivated *feline calicivirus* antigens (FCV 431 and G1 strains)	Only cats of 8 weeks or older receive the first injection; the second injection is 3 to 4 weeks later. Revaccination: the first revaccination should be carried out one year after the primary vaccination, and subsequent revaccinations: at intervals of up to three years.
	Feligen RCP (Virbac)/a modified live vaccine providing immunization of healthy cats against *feline rhinotracheitis* virus, *feline calicivirus* and *feline panleucopaenia* virus.	Cats from minimum 9 weeks of age. Two doses at an interval of 3 to 4 weeks. Annual boosters are recommended after that
Feline Leukaemia Virus	Purevax FeLV (Boehringer Ingelheim, Ingelheim am Rhein, Germany)/virus canaripox recombinante FeLV (vCP97). The vaccine strain is a recombinant canarypox virus that expresses the FeLV-A env and gag genes. Under natural conditions, only subgroup A is infectious and immunization against subgroup A induces total protection against subgroups A, B, and C. After inoculation, the virus expresses the protective proteins but does not replicate in the cat. Thus, the vaccine induces an immune state against the *feline leukemia* virus.	Cats of 8 weeks of age or older. Primary vaccination: first injection: from the age of 8 weeks. Second injection: 3 to 4 weeks later. Revaccination: annual
Feline Rhinotracheitis (Herpesvirus)	Purevax RC (Boehringer Ingelheim, Ingelheim am Rhein, Germany)/Attenuated *feline rhinotracheitis* herpesvirus (FHV F2 strain) and inactivated *feline calicivirus* (FCV 431 and G1 strains) antigens	Cats of 8 weeks of age or older. Against feline viral rhinotracheitis, for the reduction in clinical signs and against calicivirus infection for the reduction in clinical signs. Primary vaccination: first injection: from 8 weeks. Second injection: 3 to 4 weeks later. Revaccination: the first revaccination should be carried out one year after the primary vaccination, subsequent revaccinations at intervals of up to three years.
Feline Rabies	Purevax Rabies (Boehringer Ingelheim, Ingelheim am Rhein, Germany)/Contains rabies recombinant canarypox virus (vCP65); Rabisin (Boehringer Ingelheim, Ingelheim am Rhein, Germany)/inactivated rabies antigen (viral glycoproteins)	Cats 12 weeks of age and older. The cats should be revaccinated every year
Canine Rabies	Rabvac 1 (Boehringer Ingelheim Ingelheim am Rhein, Germany)/a inactivated virus vaccine; Defensor (Zoetis, Belgium SA)/ Rabico virus strain PV-Paris (*Pasteur*) replicated in a stable cell line, chemically inactivated; Rabisin (Boehringer Ingelheim, Ingelheim am Rhein, Germany)/inactivated rabies antigen (viral glycoproteins).	Rabvac 1:3 months of age or older. Revaccinate one year later and annually thereafter. Defensor: heath dogs and cats: a single dose at 3 months of age or older. Annual revaccination with a single dose is recommended. Rabisin: inactivated rabies antigen (viral glycoproteins)

Table 3. *Cont.*

Disease	Example (Supplier)/Vaccine Strategy	Recommended Vaccination Schedule
Canine distemper virus, *Canine Adenovirus* Type 2, infectious hepatitis, *Canine Parvovirus* (modified live viruses), Coronavirose canina, and Leptospira Canicola-Icterohaemorrhagiae (*L. canicola* and *L. icterohaemorrhagiae*)	V8 Nobivac® Canine (MSD, NJ, USA)/vaccine combination—modified live virus vaccine and a live attenuated vaccine	Puppies from 45 days of age, there are 3 or 4 doses in a row with intervals of 21 to 30 days between them
Canine distemper, infectious hepatitis, parainfluenza, parvovirus, coronavirus, and leptospirosis (Canicola and *Icterohaemorrhagiae serovars*), leptospirosis (Grippotyphosa and Pomona)	V10 Vanguard Plus (Zoetis, Belgium SA)/live attenuated vaccine	After V8 applications, the adult dog must be vaccinated with V10 from 6 weeks of age or older.

Nowadays, the target species focuses on vaccination schedules on species that are "almost" always directly affected; unfortunately, there is still a lack of strategies that indirectly prevent human diseases through the immunization of domestic animals and sources of infection. On the other hand, the vaccination of wild animals aimed at preventing diseases in humans or domestic animals is even more challenging and scarce. Furthermore, the primary sources of funding for research on human and animal diseases tend to be channeled to different government agencies, stifling cross-cutting approaches.

Some examples of vaccines are already available on the market and were developed to protect humans and economically valuable animals, such as Japanese encephalitis. Vaccinating horses and pigs is available, especially in countries where the disease is endemic, but, unfortunately, the costs often outweigh the benefits [39]. Other vaccines target domestic animals and aim to reduce the infection between animals and humans (as presented in Table 3).

There are a few examples of vaccines for wild animals; in this case, the objective is disease eradication and/or transmission from wild animals to humans and domesticated animals. For instance, in the State of Texas, USA, the oral rabies vaccination program led to the eradication of rabies among dog–coyote by distributing baits containing the vaccine with the aid of aircraft [40].

Some factors may suggest additional care concerning the vaccine schedules, given that there is no single ideal vaccine schedule solution for all species and regions (or countries). Instead, there are instructions, government regulations, scientific standards, professional organization guidelines, and veterinarian recommendations for vaccination programs. Any decision to adopt the vaccination schedule needs to be made on a case-by-case basis, considering the vaccination history of the animal in association with the epidemiological context of the analyzed region.

Another excellent example to illustrate the concerns transmission disease from animal to human is brucellosis. It is caused by *Brucella* spp., which are Gram-negative bacteria that have been found primarily in mammals such as goats, sheep, cattle, dogs, pigs, dolphin, porpoise, and whale, among others. Symptoms begin as an acute febrile illness with little or no localized signs and may progress to a chronic phase characterized by relapses of fever, weakness, sweating, and vague pain [41].

In cattle, the infection of *Brucella* spp. can be identified by clinical signs such as the births of weak calves, retained placenta, vaginal discharge, inflammation of the joints, and inflammation of the testicles. The most widely used vaccine for the prevention of brucellosis in cattle is the *B. abortus* S19 vaccine, but there are important differences in the dose in dependence of age and sex of cattle. The females aged 3–8 months must be

vaccinated (limited to sexually immature female animals) as a single subcutaneous dose of 5–8 $\times$ 10^{10}; however, a reduced dose of viable organisms is necessary (from 3 $\times$ 10^{8} to 3 $\times$ 10^{9}) to vaccinate adult cattle by the same administration route. Alternatively, it can be administered to cattle of any age as either one or two doses of 5 $\times$ 10^{9} viable organisms, given via the conjunctival route. It is worth mentioning that specialized veterinarians must perform the procedure due to the susceptibility of infection for those who handle it (vaccine produced by a live bacterium). To ensure the correct application of the immunizer, the veterinarian provides the vaccination certificate to the producer, which is a governmental mandatory requirement in the most of countries. Another important strategy to control and eradicate the disease is the running of brucellosis tests at least once a year, which is crucial to carry out quarantine and new exams to incorporate new the animals into the herd [41].

Factors that may influence the effectiveness of animal vaccination may be related to the vaccine (platform used in the development, interval required for application of the booster dose, addition of adjuvants in the formulation), to the host (maternal antibodies, immune system functionality, concurrent diseases, different races), to humans (storage condition, preparation, administration), and the environment (endemicity of the region and contact with strains of wild animals) [42].

In the case of bovine tuberculosis, the dose administered by the parenteral route is one hundred times lower than the dose required to ensure the effectiveness of protection by the oral route. Revaccination of cattle against tuberculosis is contraindicated, as it induces the strongest antigen-specific IFN-g responses [43].

Therefore, following the practices and protocols described in the literature and regulatory parameters is imperative. As mentioned above, each disease has a peculiarity concerning the active pharmaceutical ingredient and period to be applied in the animal's life. In addition, each country has its legislation that must be strictly followed to avoid animal and human health problems [41,44].

2. Production of Vaccines

Several methods of vaccine production have been described in the literature. The methodologies are divided into two groups denominating inactivated (killed) or live attenuated (weakened) microorganisms technologies. These techniques have been successfully used to control many diseases in the veterinary application. Each technique shows advantages and disadvantages as well as the ability to influence protective efficacy, affecting the economy of production [45].

In addition to choosing the correct strains, the qualitative composition of media used in the preparation and the production of seed cultures must be specified, namely by referring to the quality of each ingredient, and an adequate description should be registered of them. In the case of ingredients from animal origin, the species and the country of their source should be indicated and should meet the regulatory requirements. The methods used for media preparation must also be documented, including the inactivation process. The addition of antibiotics during production should usually be limited to cell cultures, inoculums injected into the eggs, and the material collected from the skin or other tissues [46].

2.1. Bacteria Seed

Bacteria used in the production of vaccines are characterized by genus and species. Whenever possible, bacteria used in production must be grown according to a seed batch system. For each primary seed batch, the origin, the date of isolation, the history of passages (including purification and characterization methods), and conservation conditions should be kept on record. Each primary seed batch should be assigned a specific identification code, whereas the minimum and the maximum number of subcultures made in each primary seed batch before the production stage should also be specified [47].

In addition, the methods used for preparing the seed crops and seed suspensions, the techniques for seed inoculation, the title and the concentration of the inoculum, and the

used means should be documented. It should be demonstrated that subcultures do not modify seed characteristics (e.g., dissociation or antigenic power). Storage conditions of each seed batch also should be documented. It must be demonstrated that each primary seed batch consists solely of bacteria of the species or the indicated strain [45].

Briefly, the method used to identify the biochemical, serological, and morphological characteristics of each strain should be registered to distinguish the strains as much as possible. Furthermore, the method applied to determine purity should also be properly registered for easy tracking if needed. If the primary seed batch contains any live microorganism other than the bacteria of the species or the indicated strain, the batch cannot be used in the production of vaccines [48].

2.2. Virus Seed

Viruses used in the production of vaccines are cultured according to a seed batch system. In this case, also for each primary seed batch, a record of the origin, date of isolation, history of passages (including the methods of purification and characterization), and storage conditions should be kept on storage and labeled with a specific code. Typically, in the production of a vaccine, the used virus must not be subjected to more than five passages from the primary seed batch. Unless otherwise indicated, the tests carried out on each primary seed batch are the ones briefly described here. It should normally not relate to microorganisms with a greater number of passages than five from the primary seed batch at the beginning of the tests [49].

The tests described below must be conducted with an appropriate volume of virus from the lysis of primary cell bank cells when the primary seed batch consists of a primary cell bank chronically infected with a virus. Appropriate tests have already been carried out in lysed cells for primary cells database validation, so it is not necessary to repeat the tests [50].

The multiplication of the primary seed batch virus and all subsequent passages must be carried out in cell culture in embryonic eggs or suitable animals to produce vaccines. Materials of animal origin must satisfy their specific requirements. A suitable method must be used to identify the vaccine strain and, as much as possible, to distinguish it from closely related strains. The primary seed batch must meet the sterility and the mycoplasmas tests. For inactivation of the complement, serum batches must be kept at 56 °C for 30 min. It must be proved that batches of serum are free of antibodies to potential contaminants of the seed virus, and they have no nonspecific inhibitory effects able to prevent infection or virus multiplication in cells (or eggs, as appropriate). If there is no possibility to use a serum with these characteristics, other methods should be used to counteract or specifically eliminate seed virus [49].

The sample of the primary seed batch should be treated with the lowest possible amount of monoclonal or polyclonal antibodies so that the virus can be neutralized as much as possible or removed [16]. The final serum–virus mixture will contain (where appropriate) a quantity of virus at least equivalent to 10 doses of vaccine per 0.1 mL or 1.0 mL, in the case of poultry vaccine or the other, respectively [51].

Next, as indicated below, the presence of foreign agents in the mixture should be investigated. For the remaining vaccines, the inoculated mixture should be at least 70 cm^2 of appropriate cell culture. Cells can be seeded in any growth phase at a lower confluence that corresponds to 70%. At least one cell of each type should be kept. Cultures should be observed daily for a week. At the end of this period, cultures are frozen and thawed three times; then, they are centrifuged to remove cell debris and re-inoculated in the same type of previous crops twice [13].

The number of cells obtained in the last passage, in suitable containers, should be sufficient to achieve the following tests [52]. Techniques, such as immunofluorescence, can be used for the detection of specific contaminants in cell cultures [53]. The primary seed batch must be inoculated in primary cells of the species origin of the virus, susceptible cells to viral pathogens for the target species of the vaccine, and sensitive cells to pestiviruses. If

the primary seed batch contains any living microorganisms other than the virus species and the indicated strain, or viral or foreign antigens, the batch cannot be used in the production of vaccines.

2.3. Computational Based Vaccine

In the last three decades, significant advances have been made in the genetic sequencing field with the use of innovative technologies such as Next-Generation Sequencing (NGS). The associated progress in the NGS area associated with the precise analysis of the sequences, the in-depth study of structural and molecular modeling and machine learning have allowed the growing interest of researchers of different areas providing the emergence of a team with interdisciplinary training, which has provided promising results in vaccinology [4] or reverses vaccinology (RV). RV is based in the rational design and development of vaccines using computational tools which identify and examine immunogenic antigens without the need for cell culture [54].

In recent years, the exponential growth of datasets with genomes of bacteria, viruses, archaebacteria, and eukaryotes has been observed, which are all freely available in databases on the web. Thus, computational techniques, bioinformatics, and immunoinformatic approaches have become essential for the better prediction and analysis of high-throughput data, aiming to identify, design, and develop new drugs or vaccines for human or veterinary and human use [55]. The main aim is the routine use of in silico techniques to favor the reduction in the time and cost of laboratory experimentation and production that generally lasts from 5 to 15 years which can also provide faster, convergent, and cost-effective discoveries of drugs [56] or vaccines [57] against new and emerging diseases.

Corynebacterium pseudotuberculosis mainly affects small ruminants such as goats and sheep. However, it can also infect horses, cattle, llamas, alpacas, and buffaloes, causing lymphadenitis clinically presented in its cutaneous, mastitis, or visceral form, which causes a significant loss in agribusiness worldwide [58]. To solve part of the problem, Soares and collaborators (2013) identified the genomic sequence of *C. pseudotuberculosis biovar equi* strain 258 to select antigenic targets and used them in reverse vaccinology to develop new vaccines for the hosts [59]. In addition, Araujo et al. (2019) also studied strains of *C. pseudotuberculosis* with the aim of in silico prospecting the development of new targets [60].

Works focused on trypanosomiasis, also known as Chagas disease, which can be caused by a protozoan of the species *Trypanosoma cruzi.* The transmission occurs through the feces that the "barber" deposits on the skin, while sucking the blood. It is endemic in South America and affects mainly humans; however, rats, dogs and cats can be a reservoir host. Ruminants are not affected. Despite efforts by different research groups, there are still no vaccines against *Plasmodium vivax*, which is one of several etiologic agents of malaria. *P. vivax* protozoan affects chimpanzees and gorillas (wild animals). In 2011, Bueno and co-authors [61] presented a selected list of antigenic and immunogenic epitopes within the Apical membrane antigen 1, which was considered the leading candidate antigens for developing a malaria vaccine. In 2020, Michel-Todó et al. published preliminary data on a rationally optimized vaccine development based on multiple epitopes of multiple antigens to neutralize the biological complexity of parasites with the aid of computational techniques for the analysis and prediction of biological data [62].

Other works have been published with a focus on the production of vaccines against brucellosis [63] and toxoplasmosis, both of which have been extensively studied with significant prevalence in humans and several animal species globally for human and veterinary use [64], having been optimally planned from reverse vaccinology with massive use of bioinformatics and computational tools.

2.4. Challenges in Vaccine Production

Viruses, parasites, bacteria, fungi, and prions are agents that cause zoonoses, all of which have extraordinarily varied life cycles and modes of transmission, providing complex

epidemiological patterns. In this context, deep knowledge of the genomic and antigenic diversity of each microorganism involved in the target disease and their epidemiological profiles are mandatory information for effective vaccine development.

Despite developing new vaccines for emerging diseases, researchers are currently addressing vaccines that can bypass inhibitory maternal antibodies, reduce dependence on the cold chain, or even adapt to husbandry management or animal owner lifestyles.

Drug delivery systems can be used to enhance the vaccine's performance, either by the slow delivery of the antigens or even by targeting specific sites. Slow delivery systems can reduce the number of doses, e.g., a vaccine that would be taken every year could be taken every two years because such systems behave like a reservoir that delivers the antigens slowly. Liposomes, lipid nanoparticles, and polymeric nanoparticles are among the delivery systems studied in veterinary vaccine development. All those delivery nanosystems have already been widely described in review papers about their application in veterinary vaccines [65–68]. Such nanoparticles can encapsulate the antigens, protect them from the body's chemical and enzymatic attacks, and even enhance the antigen's internalization into the specific body cells, improving efficiency.

Another obstacle is that most vaccines currently available must be refrigerated at 2–8 °C, and they must be protected from high temperatures as well as freezing to ensure their effectiveness. Such sensitivity is linked to the antigen used in the preparation of the vaccine, which may consist of attenuated organisms or a protein subunit, sensitive to moderate heating, or even consist of inactive organisms that are more affected by low temperatures. Such a scenario proves to be more complex when vaccines must serve herds in regions far from large urban centers, lacking the support of an adequate cold chain [69].

In addition, many of the countries endemic for diseases whose control can already be achieved using vaccines are developing, limiting investments to ensure the adequate storage and distribution of inputs in rural areas [70]. The number of vaccines for veterinary use commercially available with thermostability is still limited, such as the vaccine against Conventional Newcastle disease for chickens [71] or against rabies for dogs [72].

Although vaccination is an efficient approach to disease prevention and control, it is known that exposure of the pathogen to vaccinated animals can result in the emergence of resistant variants of the vaccine in question, with the evolution of the pathogenicity of the strain. This situation manifests itself more commonly among RNA viruses due to the high mutation rate during replication. Thus, the genome that best adapts to a given environment will prevail [73].

To ensure the effectiveness of vaccines, monitoring strategies must therefore be implemented. Adjustments in vaccination schedules or cases of resistance can thus be detected, avoiding unnecessary expenses. The immune response of the vaccinated population must be evaluated based on different indicators. In the case of foot and mouth disease, the number of outbreaks and the levels of virus circulation are determined by means of serosurveys, measuring the proportion of vaccinated animals that did not have the disease during an outbreak, compared to unvaccinated animals. It is worth noting that serological control is not sufficient to monitor the success of a vaccination program, as they are influenced by the type of vaccine and the test used to determine the antibody titer [74].

2.5. Production Methods

The vaccine production process comprises four phases: product profile, pre-development, development, registration commercial (Figure 2). The production of vaccine concentrate is characterized by the origin of the vaccine. The viral vaccines process consists of cell replication from a reference strain. The classic methodology of viral vaccine production consists in the technology of viral cultivation directly in embryonated chicken eggs free of pathogenic organisms, such as yellow fever, for example. Otherwise, the bacterial vaccines are produced by a process of fermentation of inputs and conjugation of active principles. The concentrated vaccine can only be made available for final processing after completion of the qualitative analysis, as this involves a sequence of physical, chemical, biological, and

microbiological tests that take place simultaneously. The concentrated produced vaccine is stored in cold at a suitable temperature to maintain the product's characteristics [75].

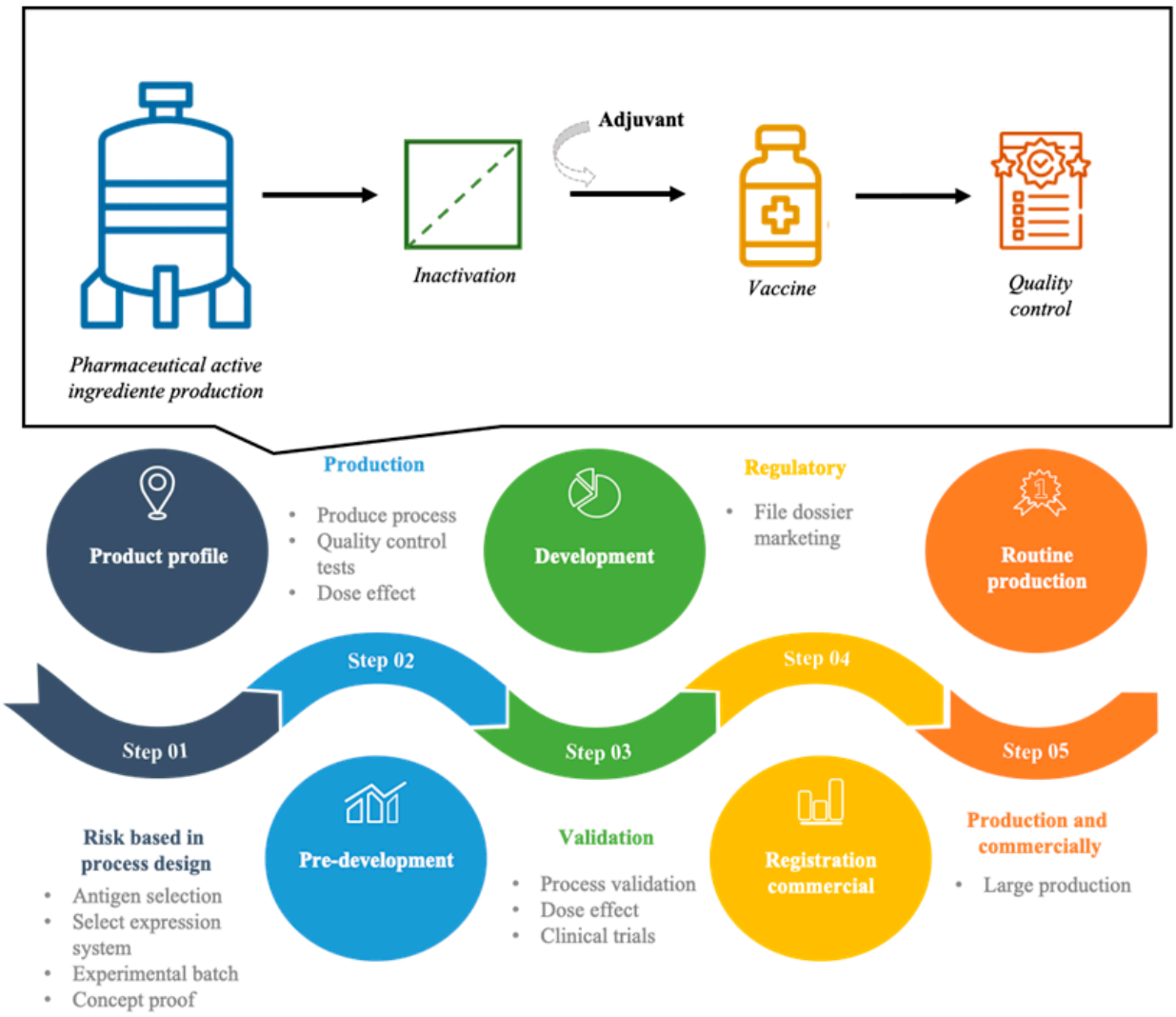

Figure 2. Steps of development vaccine from conception to production.

The active pharmaceutical ingredient (API) is the main component of the vaccine. However, other components are added to stabilize the formulation and diluting the API to the ideal fraction for veterinary application. The key adjuvants focus on improving immune response (aluminum salt), preservatives (thimerosal), stabilizers to protect against adverse conditions such as freeze and thaw (gelatin and monosodium glutamate), antibiotics to prevent contamination (neomycin, streptomycin, and polymyxin B), and microorganism suspension fluid (egg and yeast protein). As a result, you have the vaccine in bulk [76].

The final step in the process is divided into three stages: filling, lyophilization, and labeling and packaging. In the bottling, the bulk vaccine is transferred from the stainless-steel tanks to the glass bottles. The filling machine starts an in-line process of washing and sterilizing the bottles. After the vials receive the vaccine, they are closed with a butyl rubber stopper. For liquid vaccines, this closure is total, and the vials are directed via a conveyor to an aluminum cap fixing machine. The lyophilized vaccines are partially closed, and the vials are transported via trays to equipment called a lyophilizer [77].

After the freeze-drying cycle, the vials are completely closed with the stoppers they received in the filling process. When removed from the lyophilizer, the vials immediately go to a machine for applying an aluminum seal that seals each vial individually. These are

stored in a cold room separated by batches, which is followed by labeling and packaging. The completion of final processing is to package the vaccine. The vials containing the lyophilized vaccine, the liquid vaccine, or the diluent for the lyophilized vaccine are labeled with the product identification, batch number, manufacturing date, and product expiration date, among other information. Cartridges are packed in a box and then transferred to the finished products warehouse but remain in a segregated area for quarantined products until the completion of quality control and issuance of the product release certificate [78] (Figure 2).

3. Inactivation

Chemical or physical agents can carry out inactivation of virus. Among the most common inactivating agents are formaldehyde [79–83] and β-propiolactone [79,80,83,84]. Other chemical agents have also been explored, such as binary ethylenimine [80] and even natural compounds such as catechins obtained from green tea extract [85]. Green tea extract could be the first non-toxic natural compound to prepare inactivated viral vaccines with improved efficacy, productivity, safety, and public acceptance. In terms of antibody titer, cross-reactivity to heterosubtypic of viruses, and avidity to viral antigens, the quality of antibody responses to the green tea-inactivated virus was superior to that of the formaldehyde-inactivated virus [85].

Hydrogen peroxide was also used as an inactivating agent for the rabies virus. The results showed that hydrogen peroxide could replace β-propiolactone to reduce the time and cost of the inactivation process [86]. Ascorbic acid was also tested as an inactivating agent for rabies virus, but further studies are required to evaluate its effect on the cell-associated virus, probable therapeutic potential, and feasibility of replacing β-propiolactone in the production of inactivated rabies vaccine [87].

Concerning the physical inactivating agents, heat inactivation [82,88] and UV light [79] were also found in the literature. The etiological agent for Hydropericardium Syndrome (HPS) in broiler birds was inactivated by heat treatment at 56 °C for one hour and 80 °C for 10 min followed by formalin inactivation. They verified that the autogenous vaccination was extremely successful in both preventing and lowering illness in affected flocks [82]. The immunogenicity of the virus was unaffected by dual inactivation of the virus by heat and formalin treatment. Gupta et al. 1987 evaluated five inactivating methods for the diphtheria–pertussis–tetanus (DPT) vaccine [88]. Heat-inactivated pertussis (HIP) preparation was less potent than thimerosal-inactivated pertussis preparation, but the HIP was more potent than acetone-inactivated pertussis. However, HIP was similar to formaldehyde-inactivated pertussis (FIP) and glutaraldehyde-inactivated pertussis (GIP) preparations. They also checked that the inactivating agents did not affect the stability of the vaccine. On the other hand, Egorova et al. (2020) compared UV light at 253.7 nm to formaldehyde and β-propiolactone for viral inactivation during the development of a whole-virion vaccine against hemorrhagic fever with renal syndrome (HFRS). Although UV light was able to inactivate the virus, the β-propiolactone was the most promising of the tested inactivators [79].

Inactivated vaccines should be subjected to a validated process of inactivation. The described assay below for inactivation kinetics is performed only once for a given production. The other described tests are carried out in each production cycle. When the inactivation test is performed, it should have an eye out for the possibility of certain conditions of manufacture. Microorganisms can be physically protected from the inactivating agent [89].

Kinetics of inactivation must be proved if the inactivating agent and the method effectively ensure the inactivation of microorganisms in the vaccine manufacturing conditions. Data on the inactivation kinetics must be obtained. The time typically required for inactivation should not be higher than 67% of the duration of the inactivation process. If the formaldehyde is used as an inactivating agent, the test must be carried out free of formalde-

hyde [90]. To neutralize the residue of preparations of aziridine, sodium thiosulfate is added to promote the hydrolysis of this inactivating agent [91].

When using other inactivation methods, the assays must be carried out to show that the inactivating agent was eliminated or reduced to an acceptable concentration. The inactivation assay must be realized immediately after the inactivation process, or, depending on the case, after the neutralization or the disposal of the inactivating agent. If the vaccine contains an adjuvant impossible to achieve the inactivation test in the final blend inactivation, one test should be conducted during the mixture of the bulk antigen, immediately before the addition of adjuvants instead of being administered on the final batch [92].

3.1. Bacterial Vaccines

The test must be appropriate for the used bacteria and should comprise at least two passages in the culture medium used in production or, if the production is carried out in a solid medium, a suitable liquid medium or a semi-prescribed liquid in the specific monograph. The product meets the specifications if no living microorganisms are detected [93].

3.2. Bacterial Toxoids

The detoxification tests should be performed immediately after the preparation of the anatoxin and, as appropriate, after the neutralization or the elimination of the inactivating agent. The selected test should be adapted to the toxin or toxins involved, especially when in the case of sensitive assays. If there is any risk of reversion of the toxicity, one supplementary test should be performed in the earlier stage of the manufacturing process [94].

3.3. Viral Vaccines

To develop and manufacture a viral vaccine, the selection of a cell substrate is an important factor as it relies several parameters, such as cell susceptibility and permissiveness to the viral pathogen, performance in terms of viral antigens quality and production yield, primary versus continuous cells, ethical point of view, tumorigenicity status, anchorage-dependent versus suspension culture, culture medium, manufacturing cost, free of adventitious agents, and so on. Another step that has also to be considered is the format of the vaccines, as they influence the cell substrate selection, (e.g., inactivated versus live-attenuated viral vaccines; administration routes; preventive or therapeutic vaccines). The last factors to take into account are the safety and industrial considerations that deeply impact the choice of the suitable/optimal cell substrate [95].

Based on regulatory considerations, it is important make sure that all parameters are studied. These parameters included: (i) evolution of regulatory requirements for vaccine safety [96]; (ii) characterization of cell substrates used for the manufacturing of viral vaccines [97], related to, e.g., source of the cell substrate [98], history of the cell substrate [99], characteristics of the cell substrate and detection of adventitious agents, assessment of tumorigenic and oncogenic potency [100].

4. Choice of Composition and Strain of Vaccines

Among the several important aspects to be considered when choosing the composition and the vaccine strain are the safety, efficacy, and stability. Requirements to assess the safety and effectiveness have also been previously described. These requirements can be explained or supplemented by the requirements of the specific monographs. The validity must be justified by the stability studies. These comprise the titration of viruses, bacteria count and the determination of the activity. This determination is carried out at regular intervals until three months beyond the expiration date on, at least, employing three successive representative lots of vaccines stored under recommended conditions. If appropriate, the determination of moisture is also performed in lyophilized vaccines [101,102].

5. Final Bulk and Final Batch

The final bulk is formed by mixing one or more batches of the antigen, which should meet all the specified requirements, including adjuvants, such as stabilizers, antimicrobial preservatives, and solvents. The antimicrobial preservatives are used to prevent tampering or adverse-side effects caused by the vaccine microbial contamination during use. Antimicrobial preservatives cannot be incorporated in the lyophilized product. However, their use can be justified taking into account the recommended maximum duration of use of the vaccine after reconstitution, and they should be incorporated in the diluent of the lyophilized products for multiple dose [103].

Usually, the incorporation of antimicrobial preservatives in liquid preparations is not acceptable for single dose, but it may be acceptable when the same product is distributed in single-dose containers and in multiple-dose ones. In the case of multi-dose liquid preparations, the need for the use of antimicrobial preservatives must be evaluated considering the possibility of contamination during the use of the vaccine and the maximum recommended usage time after opening the container. When an antimicrobial preservative is incorporated, its efficacy must be demonstrated throughout the period of validity [104].

For inactivated vaccines, if the auxiliary substances interfere with the inactivation test, the test must be carried out for the preparation of the final bulk. This should be performed after mixing the different antigen batch but before the addition of the auxiliary substances; in case of dismissing inactivation, this should be tested at the bulk batch. Among these tests, the determination of the antimicrobial preservative free and formaldehyde, the safety test and the determination of the activity of inactivated vaccines are included [104].

As otherwise indicated in the monograph, the final bulk should be distributed aseptically into sterile containers with tamper-proof closure and sealed to prevent contamination. For the physical tests, vaccines with oil adjuvants must be submitted to the viscosity test by an appropriate method. The viscosity should be between the accepted limits for the product, and it must demonstrate the stability of the emulsion.

The chemical tests shall demonstrate, through adequate assays, that the concentrations of certain substances, such as antimicrobial preservatives and aluminum derivates, are within the set limits for the product, namely: (i) to determine the pH of liquids and diluents and demonstrate that those values lie within the limits set for the product; (ii) in certain cases, the lyophilization process is verified by determining the water content, which must comply with the approved limits for the product.

The compliance of each of the requirements prescribed in "Identification", "Test", or "Activity", and also described in the individual monographs, allows the product delivery [102,105].

6. Vaccines Assays and Quality Control

The quality of human vaccines can be evidenced by validated tests defined by regulatory agencies (WHO, FDA, EDQM, ANVISA) described in their guidelines [106–109], which defines the minimal requirements to the product. These requirements assure the products are safe and have a high quality level. However, for veterinary vaccines production, the international standard of production and quality control is described by The World Organization for Animal Health (OIE) guidelines [110]. The guidelines are discussed and prepared by VICH (International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medical Products), a trilateral program aimed at harmonizing technical requirements for veterinary product registration between the European Union, Japan, and the USA since 1996 [111].

For biological products, such as the vaccines, VICH presented guidelines to check the quality (impurities, stability, specifications) and the safety (batch safety testing and target animal safety) (Table 4).

Table 4. VICH quality guidelines for biological products.

Issue		Test	Guideline	Refs
Quality	Impurities	Test for the detection of *Mycoplasma* contamination	VICH GL34	[112]
		Test of residual moisture	VICH GL26	[113]
		Test of residual formaldehyde	VICH GL25	[114]
	Stability	Stability testing of new biotechnological/biological veterinary medicinal products	VICH GL17	[115]
	Specification	Test procedures and acceptance criteria for new biotechnological/biological veterinary medicinal products	VICH GL40	[116]
Safety	Target animal batch safety	Harmonization of criteria to waive target animal batch safety testing for inactivated vaccines for veterinary use	VICH GL50 (R)	[117]
		Harmonization of criteria to waive target animal batch safety testing for live vaccines for veterinary use	VICH GL55	[118]
		Harmonization of criteria to waive laboratory animal batch safety testing for vaccines for veterinary use	VICH GL 59	[119]
	Target animal safety	Examination of live veterinary vaccines in target animals for absence of reversion to virulence	VICH GL41	[120]
		Target animal safety for veterinary live and inactivated vaccines	VICH GL44	[121]

The impurities tests include (i) test for the detection of *Mycoplasma* contamination [112], (ii) test of residual moisture [113], and (iii) test of residual formaldehyde [114].

i *Test for the detection of Mycoplasma contamination*

Mycoplasmas are contaminants of the biological products and can be inserted by the cell culture (master seeds, stock, starting materials of animal origin). Since they can cause several disturbances, such as polyserositis, pneumonia, arthritis, otitis media and reproductive syndromes, they must be absent in vaccines [122]. The test for the detection of *Mycoplasma* contamination is apply to vaccines produced in embryonated eggs from a qualified farm. The supplier farm is responsible for the quality control of the hens, which are submitted to tests of serology for viral, avian, *Mycoplasma* and bacterial agents.

In addition, the same tests are performed by the industry quality control department [123]. In the industry, the verification of the quality of the eggshell is also performed, considering the porosity and integrity of the same in each batch of eggs supplied. The *Mycoplasma* contamination test is based on the Japan and European Pharmacopeias methods [124,125]. The vaccine formulation must be free of contaminant with *Mycoplasma* to guarantee the consistency and safety of the product. The test must be performed in working seeds and harvest seeds, starting materials (master seed, master cell seed and ingredients of animal origin) and final product. Three tests are recommended: (i) expansion in broth culture and detection by colony formation on nutrient agar plates; (ii) expansion in cell culture and characteristic fluorescent staining of DNA; (iii) nucleic acid amplification. The last one is currently approved or under consideration by regulatory authorities for more rapid detection confirmation and strain identification. This technique must be validated for inclusion in the guideline [112].

ii *Test of residual moisture (RM)*

Freeze-dried vaccines generally have RM that can impact in their shelf-life. Therefore, RM assay is applied to freeze-dried vaccines formulations. The effectivity of the freeze-dried step process is controlled by the amount of RM. The high amount of RM can interfere with the shelf life of the product; therefore, it must be limited concerning the specifications. For the determination of RM, the guideline recommends a titrimetric method (Karl Fischer), azeotropic method or gravimetric method [113].

iii *Test of residual formaldehyde*

The inactivation of botulinum neurotoxin for toxoid vaccine production occurs by formaldehyde treatment [47]. The presence of this chemical is common in inactivated vaccines. Bacterin-based vaccine (suspension of killed or attenuated bacteria) containing residual levels of formaldehyde must be analyzed by the residual formaldehyde test. The determination of the quantity of this compound refers to the vaccine safety, assuring the formaldehyde is active, it has no impact on the vaccine shelf life, and any clostridial toxoids will be antigenic and safe. The methods for the determination of residual free formaldehyde in inactivated vaccines are acetyl acetone titration, ferric chloride titration and the basic fuchsin test [114].

For new biotechnological/biological veterinary medicinal products, it is necessary to follow the stability guideline presented by VICH GL17 [115]. For this study, the selection of batches that involve drug substance (bulk material), intermediates, and drug products (finished product) is necessary for a minimum of six months after production to test their potency and purity and enable molecular characterization.

The potency tests for live and attenuated vaccine material are performed determining the number of live particles in each batch, counting or by titration. In vivo tests are required when a new seed strain is used. However, for each batch of inactivated vaccines, an in vivo potency test is required. To evaluate the purity and molecular characterization, the guideline indicated the followed methodologies: electrophoresis (SDS-polyacrylamide gel electrophoresis, immunoelectrophoresis, Western blot, isoelectrofocusing), high-resolution chromatography (e.g., reversed-phase chromatography, gel filtration, ion exchange, affinity chromatography), and peptide mapping [115]. In this step, storage conditions are also defined and controlled. The performance of the product in different temperature and humidity conditions (normal and stress conditions) is tested. The photo sensibility test may be necessary [126,127].

The specifications of procedures and acceptance criteria for new biological veterinary products to prove the adequate quality control are declared in VICH GL40 [116]. This guideline explains principles to characterize a biotechnological or biological product (determination of physicochemical properties, biological activity, immunochemical properties, purity, and impurities).

Regarding the target animal batch safety, the organization makes available three documents involving issues of Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP), Pharmacovigilance and standards for the production batch and seed batch system [117–119]. Concerning the target animal safety, the documents for live veterinary vaccines for the absence of reversion to virulence [120] and veterinary live and inactivated vaccines [121] are available.

Several methods are used to carry out the quality control of vaccines. The quality control was based on the uniqueness of each batch of vaccine. Consistency in vaccine production means that each batch of product is of the same quality and within the specifications of the batch described and effective in testing. Therefore, the development and validation of methods are crucial before the vaccine becomes a product to be marketed [128].

7. Vaccines' Labeling

The label must indicate the following: indication of the vaccine for veterinary use, the total volume and the number of doses contained in the container, the route of administration, the type or types of used bacteria or virus—in case of live vaccines, and the minimum number of live bacteria or the minimum title viruses. In the case of inactivated vaccines, the label information should comprise the minimum activity (in international units), and, if necessary, the name and the amount of any antimicrobial preservative or any other substance added to the vaccine. The presence of any substance likely to cause adverse side reactions should also be described. For lyophilized vaccines, the name, composition, and the volume of the liquid used to reconstitute the vaccine, and the time period during which the vaccine may be used after reconstitution must be present. In the case of vaccines

containing an oily adjuvant, the need for emergency medical treatment should be noted in the case of accidental injection in humans [129]. The species of animals for which the vaccine is intended should be included, in addition to the indication of the vaccine, the instructions for use as well as recommended doses for the different species.

8. Conclusions

The development and production of safe, effective, stable, and economically viable vaccines is a challenge. Over many years, the entire process has been very costly and required extensive research. Currently, the use of bioinformatic and pharmaceutical technology encompassing interdisciplinary teams that change information all over the world has reduced the time of production and development. In addition, the USA, South America, Europe and Asia have shown a large evolution in regulatory parameters connecting the product to animals through multinational industries. Veterinary vaccines are instrumental not only on animal welfare, health, and reproduction but also to human health. The COVID-19 pandemic showed that under emergency, many parties will come together to ensure that vaccines are being developed at an unprecedented speed, in addition to addressing the worldwide commercial challenges.

Author Contributions: E.B.S., A.M.S., L.M.A.M., R.d.M.B., J.F., J.C.C., A.A.d.S.-J. and C.F.d.S. contributed to the conceptualization, methodology, validation, formal analysis, and investigation, and writing—original draft, preparation. P.S., C.V., E.B.S. and R.d.M.B. contributed to the methodology, supervision, writing—review and editing, project administration, resources, and funding acquisition. All authors have made a substantial contribution to the work. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Portuguese Foundation for Science and Technology for the project UIDB/04033/2020 (CITAB), receiving financial support from FCT/MEC through national funds, and co-financed by FEDER, under the Partnership Agreement PT2020; also by the Coordenação Aperfeiçoamento de Pessoal de Nivel Superior (CAPES), Fundação de Ámparo à Pesquisa do Estado de Sergipe (FAPITEC) (PROCESSO: 88887.159533/2017-00 extração, encapsulação e caracterização de bioativos para o interesse biotecnologico) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 301964/2019-0 Chamada 06/2019, and Chamada CNPq n 01/2019). This research also was financially supported by Junta de Andalucía, under the project reference PT18 RT 3786.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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