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PRODUCTION TECHNOLOGIES, QUALITY CONTROL, CHALLENGES AND FUTURE PERSPECTIVES OF VETERINARY VACCINES

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Abstract

Veterinary diseases pose serious threats to animal health globally, particularly in endemic areas. Veterinary vaccines are necessary for animals' health, food production, animal welfare and public health. The quality of vaccines depends on manufacturing procedures, facilities, equipment and expertise. The process of vaccine formulation is crucial for the development of robust, high-quality and safe vaccines at a reasonable price. This article presents a comprehensive review of veterinary vaccines production technology, quality control, challenges and future perspectives. The vaccines specifically targeting viral and bacterial infections in livestock have been summarized. The focus is on vaccines specifically used for the control and prevention of three common viral infections i.e. Peste des Petits Ruminants (PPR), Foot and Mouth Disease (FMD), and Newcastle Disease Virus infections and three bacterial infections are enterotoxaemia, black quarter, and anthrax. The major types of viral vaccines are live attenuated, inactivated or killed, sub-unit or recombinant, toxoid, mRNA and viral vector vaccines. To increase the immunogenicity of vaccines, an adjuvant is used, in addition to stabilizers and preservatives. The process of vaccine development involves techniques like cell culture, embryonated egg inoculation, formalin inactivation, and spore preparation. Computational and bioinformatics tools are used in research and innovations of new pharmaceutical products, including vaccines. The typical adjuvants used in vaccines are aluminum salts, oil emulsions, liposomes, saponins, Toll-like Receptor (TLR) agonists, and microparticles. Although recent technological advancements have improved vaccine production is still facing several challenges, such as pathogens' complexity, understanding host pathogen genetics, identification of effective antigens, safety, stability, lack of infrastructure, re-emerging infection and cost and financial constraints. Post production quality control evaluation is necessary to ensure compliance with

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international veterinary biologics standards as recommended by the World Organization for Animal Health (WOAH).

INTRODUCTION

Several diseases impact livestock and reduce veterinary production worldwide [1,2]. The organ systems that can be commonly infected include the reproductive system, skin and urinary tract, respiratory system, digestive system, and nervous system [3]. Deadly viral and bacterial infections, such as mastitis or inflammation in the udder, reduce milk production [2,4]. It can cause severe morbidity and mortality, affecting animal health and resulting in significant economic loss [3,4]. Any illness or infection affecting domesticated animals is referred to as a livestock disease. The diseases are broadly classified into two types: Infectious diseases are caused by bacteria, viruses, parasites, or fungi and non-infectious diseases are those brought on by toxins, nutritional deficiencies, environmental stress, metabolic problems, or genetic abnormalities [2]. Infectious diseases spread quickly, cause mass mortality or long-term impact on production [5,6]. In intensively managed herds and flocks due to close contact of animals, inadequate sanitation, and a lack of biosecurity, encourages their transmission [7]. Diseases such as tuberculosis, anthrax, and brucellosis are transmitted from an infected animal to a healthy one and are known as zoonotic diseases and pose a serious public health concern [8]. Livestock diseases affect veterinary farms, resulting in substantial food insecurity in many developing countries where agriculture and livestock play an important role in the economy [9]. Due to international biosecurity restrictions and compliance requirements, trade markets and a country's export are greatly affected [10,11]

Therapeutic approaches are adopted for their treatment and control. Various antibiotics are used to treat bacterial infections. But its abuse or misuse contributes to the emergence of antimicrobial resistance (AMR), a serious worldwide concern in veterinary medicine [12]. In many regions, livestock treatment is less accessible, more expensive, and less sustainable. These issues highlight the significance of preventative health strategies, especially in small-scale farmers. Vaccination is the cheapest and most successful preventive strategy to control the prevalence of endemic and pandemic diseases [13]. Similarly, immunization decreases the spread of disease outbreaks, promoting herd immunity and reducing the need for antibiotics [14,15]. The veterinary vaccines supply depends on imported biologics in low setting areas, which are expensive, not frequently available, and not specially made for locally circulated strains of pathogens [5]. The production of strain-relevant vaccines ensures accessibility, consistency, and captures the attention of the researchers [16]. The selection of high-quality biologics, particularly for more common livestock infections, is usually performed by the institutions that design the vaccines [17]. There are various types of bacterial vaccines that are used for the control of bacterial diseases outbreaks including black quarter, enterotoxaemia, and anthrax. The antiviral vaccines are used for the prevention of Newcastle disease virus, Peste des Petits Ruminants (PPR), and Foot and Mouth Disease (FMD) virus [8]. Quality assurance of vaccine production is necessary to maintain sterility, safety, potency, and antigens' identity confirmation [18,19]. These should be performed under the instructions of world regulatory requirements [20].

Developing methods for most veterinary vaccines still relies on a classical strategy with live pathogens that possess a strong immunogenicity either with high virulence or without virulence. Other than that, in developing countries, vaccine production is still facing several challenges, such as financial issues, deployment of conventional methods, and poor infrastructure [21]. The opportunities of technical modernization and increased self-reliance in veterinary vaccine manufacturing are still being created by academic engagement and international alliances [13].

An overview of veterinary vaccine production techniques and related quality assurance procedures is summarized. It provides an outline of the main processes utilized in the production of significant bacterial and viral vaccines. In order to

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address context-specific disease burden, improve self-reliance, and lessen reliance on imported biologics, we provided an insight into the wider significance of bolstering local and regional vaccine development capability.

Types and production of veterinary Vaccines

Two main type of vaccines which are frequently mentioned and of a great importance are bacterial vaccines and viral vaccines [16,22]. Viral vaccines can be categorized as live attenuated vaccines and inactive or killed vaccines. Other type of viral vaccines includes recombinant or sub-unit or conjugate vaccine, toxoid, viral vector, mRNA, and DNA vaccines [23,24]. The live attenuated or inactivated vaccines are processed by propagating viruses in embryonated eggs or cell culture, commonly used cell lines include Vero cell lines and BHK 21 cell lines. Live attenuated or inactivated vaccines are prepared by the propagation of virus in embryonated eggs for vaccines against Newcastle Disease Virus (NDV), or in mammalian cell cultures, such as vero cell lines or BHK-21 cell lines for vaccines against Foot and Mouth Disease (FMD), and *Peste des Petits Ruminants* [19,25]. Likewise, antibacterial vaccines for the control of enterotoxaemia and black quarter are made by growing pathogens under laboratory conditions in nutrients-enriched anaerobic media [3,26]. To boost the immunogenicity of the vaccine and maintain the structural integrity during storage adjuvants like alum or hydroxide gel are added in the vaccine. Skimmed milk or gelatin is used for lyophilization. For the prevention of contamination by microbes, many preservatives, including thiomersal, are used [27]. The major differences between viral and bacterial vaccines are shown in Table 1.

Table 1. Comparison of viral and bacterial vaccines

Characteristics	Vaccine types	
	Viral vaccines	**Bacterial vaccines**
Used against	Virus	Bacteria
Propagation system	Embryonated eggs (NDV), Cell culture (Vero for PPR, BHK21 for FMD)	Anaerobic liquid media (RCM broth, liver-meat broth)
Vaccine type	Live attenuated or inactivated virus	Inactivated whole-cell or toxoid vaccines
Adjuvant	Sometimes adjuvants used (e.g., alum in inactivated FMD)	Alum, aluminum hydroxide gel (especially in toxoid/bacterin vaccines)
Preservatives	Thiomersal (multi-dose vials)	Thiomersal (standard concentration 0.004–0.01%)
QC test	EID_{50}, $TCID_{50}$, sterility, ELISA, PCR, CPE observation	Sterility, safety (in animals), potency (in vivo or pH testing)

Depending on the viral strain and host specificity, mammalian cell culture techniques or embryonated eggs are frequently used to manufacture several important viral veterinary vaccines [19,25]. These include vaccines against Foot and Mouth Disease (FMD) in cattle and other animals with cloven hooves, Peste des Petits Ruminants (PPR) in small ruminants, and Newcastle Disease Virus (NDV) in poultry. Every viral vaccine is subjected to a thorough quality control review, which includes sterility and safety testing in laboratory animals or verified cell lines, as well as potency determination utilizing tests like EID_{50} or $TCID_{50}$ [3]. Vaccines can be designed as chemically inactivated biologics (e.g., FMD) or live attenuated formulations (e.g., NDV Lasota and PPR), depending on the pathogen and vaccination approaches. Discrete formulation components, like stabilizers and preservatives are added into the

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vaccine while synthesis to maintain the structure, integrity, immunogenicity [27].

Newcastle Disease Vaccine

Different species of birds, peculiarly poultry, are highly susceptible to contagious viral infections [19,28]. The level of severity and symptoms are determined by the type of viral strain for instance, the signs for *Avian Paramyxovirus* serotype-1 include discomfort in respiratory system, decrease in egg production, neurological distress, and morality [29]. To prevent New Castle disease virus, two type of vaccines have been prepared for both lentogentic Lasota strain and mesogenic Mukhteswar strain. The Lasota strain vaccines are live attenuated and lentogenic (less virulent) and should be administered to young chicks on a regular basis [16,30]. Whereas, the Mukteswar strain is a mesogenic (moderate pathogenicity) vaccine that is administered to older birds to boost the immunogenicity [19]. To obtain a sterile, nutrient rich, and ideal environment for viral replication, few days old pathogen free embryonated eggs are used for the propagation of both vaccines [3,31]. Manufacturing process is started when, each embryonated egg's air sac is carefully inoculated with viral seed under strict aseptic conditions [2]. Following injection, the eggs are candled regularly to track the viability of the embryos throughout a 4-day incubation period at 37°C [19]. After incubation, eggs with live embryo are selected for harvesting and allantoic fluid with abundant viral titers is collected [16,32]. Afterwards, the liquid is mixed with skim milk, which acts as a stabilizer and protects the virus against lyophilization [33]. Antibiotics such as streptomycin, gentamycin, or penicillin, are added to prevent any bacterial growth and further contamination [33]. The potency of vaccine is assessed by EID_{50}, the dilution at which injected embryos are 50% infected [19].

To make a robust immunological response, this test is conducted in serial dilution. Depending on the type of strain and manufacturer guidelines, the maximum acceptable potency for Newcastle Disease Virus vaccines typically ranges from 10^3 to 10^6 EID_{50} per dose [16]. The chicks are given the Lasota strain via ocular way or drinking water to give preliminary defense against disease during early growth phase [19]. A second dosage is provided at 3-4 weeks of age, particularly to breeder flocks, and the third booster dose is administered at 16 weeks. Due to its high virulence, the Mukhteswar strain is inoculated in birds which are older than six weeks [33].

Prior to field administration, the Newcastle Disease vaccine is passed through quality control testing, which includes identity confirmation using techniques like hemagglutination inhibition (HI) tests, safety testing in laboratory animals (e.g., mice or chicks), potency assessment using EID_{50}, and sterility testing using bacterial and fungal culture media [19]. This disease is continuously a huge threat to the chicken's health and productivity in various areas where poultry industry has strong economic impact [16]. To prevent the disease in chicken population, control disease outbreak and develop long term immunity the creation and accessibility of widely used vaccine strains, including Lasota and Mukteswar, are crucial. The use of vaccine in veterinary industry enhances productivity and health while contributing to economic gains for producers in the poultry industry [21].

Peste des Petits Ruminants (PPR) vaccine

Morbillivirus is the causative agent of *Peste des Petits Ruminants*, this virus belongs to the family *Paramyxoviridae* [34,35]. The small ruminants, like sheep and goats, are susceptible to PPR [36]. The common symptoms include respiratory distress, fever, diarrhea, oral and nasal discharges, and mortality, particularly in juvenile animals. Due to severe economic impact and transboundary dissemination, it gained special eradication attention from the PPR Global Eradication Programme (GEP), which is jointly run by the Food and Agriculture Organization (FAO) [37,38]. The live attenuated vaccines, especially those based on internationally recognized Nigerian 75/1 strain, are commonly used for their control because of safety, durability, and potent immune response [2,19].

The cells of African green monkey kidney are highly sensitive and an ideal site for multiplication of morbilliviruses [19,39]. The cells are kept in a

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sterile culture containing Glasgow Minimum Essential Medium (GMEM) supplemented with fetal bovine serum (FBS) under aseptic conditions, which promotes ideal growth [33,40]. Once the confluent monolayer is formed, the attenuated PPR virus is then transferred under biosafety level 2 containment conditions [33]. Cytopathic effects such as cell rounding, detachment, syncytia formation and clumping shows active infections and are used to track viral replication patterns under a microscope [16,35]. The intracellular virus particles are then released by freezing and thawing the infected cell culture [27]. The captured viral suspension is then filtered to make it clear and is mixed with stabilizers like skim milk to maintain the structural integrity of virus during lyophilization [33]. Then, after freezing vaccines are vacuum-sealed in a sterile glass vial and stored at 2-8 °C to extend its shelf life [19,41].

Before field distribution, each vaccine batch is subjected to rigorous quality control testing to assess the potency, safety, sterility, and necessary antigenic load [33,42]. A 1mL subcutaneous dose is normally injected at 3-4 months as part of the standard field administration to develop robust immunity [37]. A single dose of vaccine can provide safety and immunity has been shown to extend to three years [35]. This makes it suitable for global vaccination programs in areas of poor veterinary infrastructure. Due to these features, attenuated PPR vaccines are crucial for global eradication initiatives of PPR, providing a stable and inexpensive means of disease control in small ruminant populations worldwide [37].

Foot and Mouth Disease Vaccine

Foot and Mouth disease is one of the most economically devastating diseases infecting cloven-hoofed animals such as sheep, goats, cattle, buffalo, and pigs [19,43]. The Foot and Mouth Disease Virus (FMDV) is a highly transmittable virus belonging to the Genus Aphthovirus and family Picornaviridae (Grubman & Baxt, 2004; Raoof, 2024). The symptoms include fever, vesicular lesions in feet, teats, and mouth, drop in milk production, salivation, and lameness [2]. It is a transboundary veterinary disease that should be reported due to its quick dissemination and high morbidity rate [33]. In endemic areas, immunization is commonly used to control the numerous serotypes of circulating virus [6,19]. The Baby Hamster Kidney (BHK-21) cell lines are highly suitable for FMD virus (FMDV) replication and producing high viral yields used in vaccine design [44]. The cells are kept in Glasgow Minimum Essential Medium (GMEM) and then supplemented with fetal bovine serum (FBS) and antibacterial agents to promote growth and avoid contamination [16]. The selected serotype is inoculated in biosafety level 3 (BSL-3), in accordance with international recommendations for managing high-risk infections [33]. Cytopathic effects (CPE), such as detachment and rounding of cells, are used as indicators while observing viral replication under microscope [44].

Once the standard titers are obtained, the culture is harvested. The binary ethylenimine (BEI) is used for the removal of infectivity, while Glasgow Minimum Essential Medium (GMEM) is supplemented with fetal bovine serum (FBS) and antibacterial agents' immunogenic epitopes required for protective immunization. It is used to chemically inactivate the viral suspension and assure vaccine safety [19]. Following this, binary ethylenimine is neutralized by adding sodium thiosulfate and filter it to make it clear [33]. Adjuvants are added to boost the immunogenicity. Aluminum hydroxide gel encourages gradual antigen release and saponin acts as an immunopotentiator [27]. Thiomersal (0.01%) is commonly used as preservative against microbial contamination, especially in multi-dose formulation [16,46]. Each batch of vaccine is then subjected to an extensive quality control testing before distribution. These include testing the safety, infectivity assessment, antigen quantification, and sterility testing to rule out any bacterial and fungal contamination [33].

Administration of these vaccines is scheduled for every two years depending on different risk levels such as, geographical distribution, epidemiological characteristics, and animal movement patterns [19]. A significant decrease in outbreaks and viral circulation has been noticed in endemic population after repeated immunization with inactive FMD vaccine [8,16]. Cell culture-based

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inactivated vaccines are an essential tool for achieving long-term FMD control and eventual eradication goals when paired with cold-chain compliance biosecurity precautions, and molecular surveillance [16].

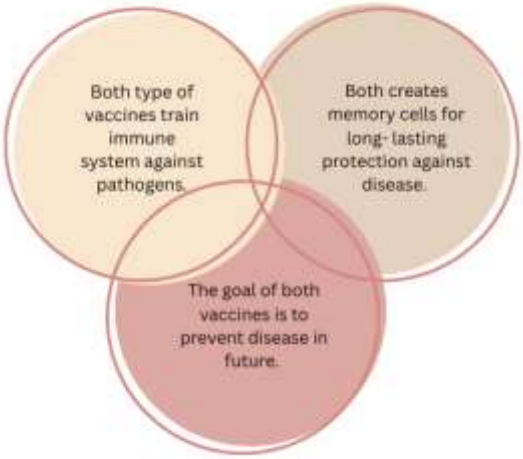

Figure 1. Similarities between viral and bacterial vaccines.

Bacterial Vaccines

The bacterial vaccinations are an integral part of livestock illness preventive strategies [2]. There main target is anaerobic, spore-forming, and toxic bacterial pathogens, these pathogens or toxins can infect livestock animals such as sheep, cattle, buffalo and goats, causing grave infections which can sometimes prove fatal. [19,47]. Vaccines manufactured against enterotoxaemia, black quarter (BQ), and spore-based anthrax-like illnesses are important examples. They are usually manufactured as toxoid or inactivated vaccines and like viral vaccines they also include adjuvants to boost immunogenicity and provide antigen integrity [16].

Enterotoxaemia Vaccine

Enterotoxaemia is also known as pulpy kidney disease, it is caused by the overgrowth of *Clostridium perfringens* type D. This condition mainly affects sheep and goats and can sometimes be fatal [2]. This Gram-positive, spore-forming, anaerobic bacterium produces a highly strong epsilon toxin that causes intestinal damage, neurological symptoms, and sudden death, particularly in young and rapidly growing animals [48]. This results in substantial financial loss in small-scale ruminant farming and is prevalent in flocks that are either unvaccinated or under-vaccinated [16]. *Clostridium perfringens* type D is first cultivated anaerobically in Reinforced Clostridial Medium (RCM) broth, a nutrient-rich medium that is perfect for clostridial development [19]. To promote ideal bacterial growth and toxin production, the culture conditions are carefully maintained, usually at 37°C, with an oxygen-free environment and pH management [33]. For the activation of epsilon protoxin into its poisonous form trypsin is added to the culture. Once adequate bacterial growth is achieved, it is a crucial step for the conversion of toxoid [48].

Formaldehyde solution (0.4%) is added into the toxin to inactivate its pathogenic effect but the antigenicity is maintained throughout the process, it is then cultured for 5-7 days for adequate growth [16]. These inactivation procedures are done to ensure the non-toxicity of the vaccine and preserve the immunogenic elements necessary to trigger an effective immune response [33]. Aluminum potassium sulfate (alum) is then added to the inactivated toxoid to improve its antigenic presentation and extend the immune response [27]. Usually, thiomersal is added at a concentration of 0.01% to ensure the stability of

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the vaccine during storage [16]. The final vaccine is sealed in a bottle under sterile conditions and then passed through extensive quality control testing, pH measurement (usually 6.5-7.5), safety testing in laboratory animals to confirm the absence of adverse reactions, sterility testing to ensure that there is no microbial contamination, and finally potency testing, which may involve in vivo trials or antigen quantification to verify protective efficacy [19]. For sensitive animals, especially lambs and juveniles, the normal field dose is 2 ml given subcutaneously. A booster shot is advised every year or as directed in high-risk flocks [2]. Early vaccination is highly important for susceptible and quickly growing animals([48]. The manufacturing of these vaccinations is essential for preventing unexpected fatalities in small ruminants, reducing the need for antibiotics, and improving livestock financial issues in rural and resource-constrained environments [16]

Black Quarter Vaccine

Clostridium chauvoei is a Gram-positive, spore-forming, anaerobic bacteria, which cause Black Quarter (BQ) disease, also called Blackleg. It is an acute and deadly disease affecting cattle and buffalo [2]. This results in mortality within 24-48 hours and is characterized by an abrupt onset of fever, lameness, acute muscle swelling, and fast necrosis [48]. Soil contains *C. chauvoei* spores, which can enter the body through wounds or ingestion, particularly during the monsoon season when animals graze in muddy or wet places [19]. Vaccination is the most effective means of management because of its quick progression and poor responsiveness to treatment [16]. The Black Quarter vaccine is a formalin-inactivated bacterin that exposes the animal's immune system to *Clostridium chauvoei's* conserved surface antigens in order to produce immunity [19]. These can be produced in an anaerobic environment, nutrient-rich medium, such as meat peptone broth or liver infusion broth, under regulated temperature [33]. Formalin (formaldehyde) is applied to the culture to inactivate the pathogen when optimal bacterial growth has been reached [19]. This phase guarantees that the bacteria are degraded while maintaining the structural integrity of immunogenic surface proteins [48]. A commonly used adjuvant, aluminum hydroxide gel, is combined to improve the immune response by extending the release of antigens and encouraging antigen absorption by immune cells. This is now known as a bacterin [27]. The vaccine formulation is conducted in a sterile environment with proper adjustment of pH (usually 6.8-7.2) [33].

The vaccine is then subjected to extensive quality control testing prior to field administration. These tests include sterility tests, safety testing in lab animals, stability tests, visual inspection, and viscosity checks to evaluate product consistency and uniformity [19]. In the recommended field, approximately 2 mm should be applied subcutaneously in different regions, usually in the neck [16]. The vaccination is administered once a year, ideally prior to the monsoon season, because Black Quarter outbreaks are most likely to occur due to elevated environmental spore activity [2]. Booster shots are administered or repeated every year in endemic areas [33]. In rural and semi-urban regions, Black Quarter continues to pose a serious concern. So, the availability of these vaccines safeguards the livestock [16].

Anthrax Vaccine

Bacillus anthracis is a rod-shaped, Gram-positive, spore-forming bacterium that can persist for decades in polluted soil because it can produce extremely resistant endospores. It causes a dangerous zoonotic disease known as anthrax [2]. It infects cattle, buffaloes, sheep, goats, and humans as well [49]. The disease can be manifested as acute or per-acute, and frequently results in massive bleeding, bloody discharge from bodily openings, and sudden death [19]. Anthrax is a communicable disease, and vaccination is necessary in endemic regions [33]. A toxic but non-capsulated strain of *Bacillus anthracis* called Sterne strain is used to make anthrax vaccine [19]. The bacterium is attenuated and safe for use in animals while maintaining its immunogenic properties because it cannot elude the host's immune system due to the absence of the capsule [16] By actively infecting the host's immune system with viable but non-virulent spores, this live spore

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vaccine is intended to produce long-term immunity [19].

The first step in the production procedure is to cultivate the *Bacillus anthracis* Sterne strain under stringent aerobic conditions in large-capacity Ross flasks filled with sporulation-enhancing media [33]. To enable optimum sporulation, the culture is incubated for a long time, usually 30 to 40 days [16]. The bacterial culture is harvested and processed to produce a spore-rich suspension appropriate for vaccine formulation once sufficient spore growth has occurred [19]. The standardized spore suspension is then suspended in glycerol saline which can serve as a diluent to preserve spores' viability throughout storage and transfer [16]. It is bottled and sealed under sterile conditions and kept at 4-8 °C. In cold chain maintenance the vaccine has a shelf life of almost one year [33]. Anthrax vaccination does not contain adjuvants or preservatives because it is a live spore vaccine, in comparison to toxoid or inactivated bacterial vaccines [49]. The spores are the source of immunogenicity and provide protective immunity by eliciting humoral and cellular immunological responses, 5-7 days after vaccination [2]. Once a year a field dose of 1 ml should be administered subcutaneously in cattle, buffaloes, sheep, and goats in high-risk zones like lowland or flood-affected zones [16].

Precautionary recommendations are strict such as vaccinating healthy animals, avoiding vaccinations during outbreak of any pandemic or when animals are ill, stressed, or immunocompromised, ensuring the hygiene of injection site and equipment sterilization to prevent infection [19]. Vaccine manufacturing should be carried out under biosafety level 3 (BSL-3) [33]. This vaccine is vital for the prevention of anthrax outbreaks and economically beneficial to prevent livestock mortality [16].

Quality Control Testing

In order to ensure the quality and standard of vaccine each batch is subjected to ``specific requirements for safety, sterility, potency, and identity testing prior to release, quality control (QC) is an essential part of vaccine production units [19]. Systematic testing of both viral and bacterial vaccines is necessary to ensure their quality. Ensuring the consistency of products and regulatory compliance with globally recognized biosafety standards and manufacturing protocols are crucial aspects of quality control [33].

The fundamental aspect of quality control unit includes sterility testing, to confirm the absence of any microbial contamination in the vaccine [16]. Varied culture media, including Nutrient Agar, Sabouraud Dextrose Agar (SDA), MacConkey Agar, Thioglycollate Broth, and Fluid Thioglycollate Medium, are employed to detect aerobic and anaerobic bacteria as well as fungi [19,50]. During this period, if visible microbial growth is detected it indicates contamination and necessitates batch rejection or reprocessing [16]. After being inoculated into this medium, vaccine samples are incubated for up to 14 days at controlled conditions.

Small doses of the vaccine are given to laboratory animals, such as mice, guinea pigs, or rabbits, to test the level of safety and observe toxicity [2,49]. The identity testing is required to ensure that the product contains the right antigen or microbe. Various methods, including Gram staining, ELISA, PCR, or serological cross-matching, are used to guarantee that the vaccines are free from microbial contamination [16,33]. To access the level of potency of a vaccine, assays like EID_{50} (Embryo Infective Dose 50) or $TCID_{50}$ (Tissue Culture Infective Dose 50) are employed [19]. In these tests, serial dilution and titration are conducted in tissue cultures or embryonated eggs [33]. The potency is evaluated for toxoid vaccines, using either serological techniques or in vivo challenge testing in vaccinated animals [2,16]. Additional tests such as pH measurement and antigen quantification are performed to ensure homogeneity of each batch [50].

Vaccine production should be conducted under strict biosafety regulations. These include proper equipment such as an autoclave for instrument sterilization, class II biosafety cabinets to handle live organisms, and disinfectant to keep the environment clean and free from microbial contamination [49]. All biological waste should be disposed of by proper waste management protocols and purified water should be used for

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culture media preparation [33]. Personal training on aseptic procedures, pathogens containment, and emergency response is also essential to minimize laboratory-acquired diseases and risk management [16].

Vaccines are safe and effective methods of disease control and prevention, and comply with both national and international standards. Following the above measures will improve animal health and increase trust among farmers, veterinarians, and legislators on the suggestion of locally manufactured veterinary biologics [33]. For the development of veterinary vaccines guidelines and protocol set by the International Organization for Standardization (ISO) and World Health Organization. (WHO) must be followed [50,51].

Challenges and Future Perspectives

Despite notable advancements, the production of veterinary vaccines is still facing several problems [16,52]. The manufacturing of these biologics is impacted by technical and regulatory constraints [33]. To consummate the increasing demand of livestock industry, which is facing many challenges such as emerging diseases, abiotic resistance, and transboundary epidemics, it is vital to address barriers [19,53]. The use of conventional vaccine manufacturing methods, such as formalin-based inactivation and embryonated egg injection, is a significant hindrance. Although these procedures work well, they are time consuming and require attention to detail [16]. Lack of properly trained staff and unavailability of proper resources hamper the production of vaccines [49,50,54].

International collaboration, and extensive policy support, are necessary to manufacture standard vaccines [33]. Finding innovative techniques, newer adjuvants, novel delivery methods, and thermostable formulations is hampered by a lack of funding and resource management [16]. Investing in cutting-edge biological platforms, laboratory facilities and staff training is essential for the better future of veterinary vaccine industry [50]. Vector-based vaccinations, RNA vaccines, and nanoparticle-based delivery approaches can be accelerated by collaboration with industry and a global research network [33]. Similarly, improving food security, disease control, animal welfare, and economic resilience on livestock can overcome these obstacles [16,55]

Conclusion

The control of infectious diseases in livestock is greatly reduced by antiviral and antibacterial vaccination. The development of important biologics, such as vaccines against Newcastle Disease Virus (NDV), Peste des Petits Ruminants (PPR), Foot and Mouth Disease (FMD), enterotoxaemia, Black Quarter, and spore-based anthrax-like illnesses, is necessary for the control of pandemics and epidemics. To synthesize standards, vaccines, biologics, and quality control is highly mandatory, such as sterility, potency, safety, and identity testing. Vaccine production is facing several challenges despite advanced technologies, such as resource constraints, unavailability of equipment, unreliable cold chain systems, and many more. Cutting-edge technology and great improvement are necessary to control the contagious diseases in the veterinary sector. Industrial partnership, national and international collaboration are crucial. Vaccinations reduce the chances of misuse of antibiotics and act as prophylactic measures. Disease control will increase livestock productivity and badly impact a country's economy.

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Conflict of interest

The authors declare no conflict of interest.

References

[1] Capper JL. The impact of controlling diseases of significant global importance on greenhouse gas emissions from livestock production. One Health Outlook 2023; 5: 17. doi:10.1186/s42522-023-00089-y

[2] Radostits OM, Gay CC, Hinchcliff KW, et al. A textbook of the diseases of cattle, horses, sheep, pigs and goats. Vet Med 2007; 10: 2045–2050

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

[3] OIE. OIE standards and guidelines for veterinary vaccines: WOAH. 2021. Retrieved from https://www.oie.int. Accessed: 11 December 2025.

[4] Cheng WN, Han SG. Bovine mastitis: Risk factors, therapeutic strategies, and alternative treatments—A review. Asian-Australas J Anim Sci 2020; 33: 1699

[5] Khan FA, Khan MA, Shabbir MZ, et al. Vaccine production and quality control in Pakistan: Status, challenges, and prospects. Pak Vet J 2022; 42: 245-250.

[6] Mahapatra M, Parida S. Foot and mouth disease vaccine strain selection: current approaches and future perspectives. Expert Rev Vaccines 2018; 17: 577–591. doi:10.1080/14760584.2018.1492378

[7] Okoth E, Ochieng L, Njehu A, et al. Dairy cattle herd health management manual for animal health service providers. 2024;

[8] Singh RK, Sharma GK, Mahajan S, et al. Foot-and-mouth disease virus: immunobiology, advances in vaccines and vaccination strategies addressing vaccine failures—an Indian perspective. Vaccines 2019; 7: 90

[9] World Bank. Livestock in Pakistan: Opportunities and challenges (Report No. 97828-PK). 2020. Retrieved from https://documents.worldbank.org. Accessed: 12 December 2025.

[10] De Clercq K, Mackay DKJ. Regulation of FMD vaccines within the European Union. In Report of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease. Food Agric Organ Rome Press 2002;

[11] Hammoudi A, Grazia C, Surry Y, et al., Hrsg. Food Safety, Market Organization, Trade and Development. Cham: Springer International Publishing; 2015

[12] Palma E, Tilocca B, Roncada P. Antimicrobial resistance in veterinary medicine: An overview. Int J Mol Sci 2020; 21: 1914

[13] Schijns V, Fernández-Tejada A, Barjaktarović Ž, et al. Modulation of immune responses using adjuvants to facilitate therapeutic vaccination. Immunol Rev 2020; 296: 169–190. doi:10.1111/imr.12889

[14] Bahri H, Awais A, SaidMiraj Ud D, et al. CHAPTER NO. 5 DEVELOPMENT AND THERAPEUTIC POTENTIAL OF SMALL MOLECULE IMMUNOMODULATORY FOR TREATING DIFFERENT IMMUNE-RELATED CONDITION. Trends Pharma Sci 2024; doi:10.5281/zenodo.14281632

[15] Rojas JM, Martín V, Sevilla N, et al. Vaccination as a Strategy to Prevent Bluetongue Virus Vertical Transmission. Pathogens 2021; 10. doi:10.3390/pathogens10111528

[16] FAO. Food and Agriculture Organization of the United Nations: Livestock health and food security. Retrieved from https://www.fao.org. Accessed 12 December 2025.

[17] Marzouk E, Alajaji AI. Preventive Immunology for Livestock and Zoonotic Infectious Diseases in the One Health Era: From Mechanistic Insights to Innovative Interventions. Vet Sci 2025; 12: 1014

[18] Barbosa R de M, Silva AM, Silva CF da, et al. Production technologies, regulatory parameters, and quality control of vaccine vectors for veterinary use. Technologies 2022; 10: 109

[19] OIE. World Organisation for Animal Health. OIE Annual Report 2018. Paris. 2018. Retrieved from https://rr-europe.woah.org/en/news/oie-annual-report-2018/. Accessed 14 December 2025.

[20] WAOH. World Organisation for Animal Health (WOAH) Report. Implementation of WOAH standards: The Observatory Annual Report 2022. 2022. https://www.woah.org/app/uploads/20

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

22/12/annual-report-observatory-2022.pdf. Accessed: 10 December 2025.

[21] Lee N-H, Lee J-A, Park S-Y, et al. A review of vaccine development and research for industry animals in Korea. Clin Exp Vaccine Res 2012; 1: 18–34

[22] Kahn LH, Bergeron G, Bourassa MW, et al. From farm management to bacteriophage therapy: strategies to reduce antibiotic use in animal agriculture. Ann N Y Acad Sci 2019; 1441: 31–39. doi:10.1111/nyas.14034

[23] Bohara VS, Kumar S. Viral Vaccines. In: Textbook of General Virology. CRC Press; 2025: 182–188

[24] Pöri P. Development of vaccines. 2018;

[25] Rodrigues AF, Soares HR, Guerreiro MR, et al. Viral vaccines and their manufacturing cell substrates: New trends and designs in modern vaccinology. Biotechnol J 2015; 10: 1329–1344. doi:10.1002/biot.201400387

[26] Kushram N, Sharda R, Misraulia KS, et al. Production and standardization of combined vaccine against haemorrhagic septicaemia and black quarter. Int J Curr Microbiol Appl Sci 2020; 9: 2534–2541

[27] Srivastav AK, Das P. To study the production and standardization of veterinary vaccines. Int J Sci Res 2015; 4: 2331–2337

[28] Brown VR, Bevins SN. A review of virulent Newcastle disease viruses in the United States and the role of wild birds in viral persistence and spread. Vet Res 2017; 48: 68. doi:10.1186/s13567-017-0475-9

[29] Reid SM, Skinner P, Sutton D, et al. Understanding the disease and economic impact of avirulent avian paramyxovirus type 1 (APMV-1) infection in Great Britain. Epidemiol Infect 2023; 151: e163

[30] Wajid A, Basharat A, Bibi T, et al. Comparison of protection and viral shedding following vaccination with Newcastle disease virus strains of different genotypes used in vaccine formulation. Trop Anim Health Prod 2018; 50: 1645–1651. doi:10.1007/s11250-018-1607-6

[31] Guan L, Ping J, Lopes T J, Fan S, Presler R, Neumann G, & Kawaoka Y. (2023). Development of an Enhanced High-Yield Influenza Vaccine Backbone in Embryonated Chicken Eggs. Vaccines, 11(8), 1364.

[32] Santry LA, McAusland TM, Susta L, et al. Production and purification of high-titer Newcastle disease virus for use in preclinical mouse models of cancer. Mol Ther Methods Clin Dev 2018; 9: 181–191

[33] WOAH. World Organisation for Animal Health. OIE standards and guidelines for veterinary vaccines. 2021. Retrieved from https://www.oie.int. Accessed: 8 December 2025.

[34] Bailey D, Banyard A, Dash P, et al. Full genome sequence of peste des petits ruminants virus, a member of the Morbillivirus genus. Virus Res 2005; 110: 119–124

[35] Diallo A. Peste des petits ruminants. Revue Scientifique et Technique de. Off Int Epizoot 2003; 22: 967–976. doi:https://doi.org/10.20506/rst.22.3.1413

[36] Ahaduzzaman M. Peste des petits ruminants (PPR) in Africa and Asia: A systematic review and meta-analysis of the prevalence in sheep and goats between 1969 and 2018. Vet Med Sci 2020; 6: 813–833. doi:10.1002/vms3.300

[37] FAO, WOAH. Peste des petits ruminants global eradication programme: Contributing to food security, poverty alleviation and resilience (2017–2021). 2016

[38] Fayyad AF, Alzuheir IM. Peste des petits ruminants: past, present, and future scope. J Infect Dev Ctries 2024; 18: 1837–1845

[39] Begum S, Nooruzzaman M, Hasnat A, et al. Isolation of peste des petits ruminants virus using primary goat kidney cell culture from kidneys obtained at slaughter. Vet Med Sci 2021; 7: 915–922. doi:10.1002/vms3.413

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

[40] Abbas F, Ullah A, Awan MA, et al. Production of tissue culture based peste des petits ruminants (PPR) vaccine at CASVAB, Quetta, Pakistan. Pak J Life Soc Sci 2012; 10: 80–83

[41] Hensley C, Zhou P, Schnur S, et al. Thermostable, dissolvable buccal film rotavirus vaccine is highly effective in neonatal gnotobiotic pig challenge model. Vaccines 2021; 9: 437

[42] Saravanan P, Sen A, Balamurugan V, et al. Rapid quality control of a live attenuated Peste des petits ruminants (PPR) vaccine by monoclonal antibody based sandwich ELISA. Biologicals 2008; 36: 1–6

[43] Soomro H, Hassan MF, Rajput ZI, et al. Foot and Mouth Disease (FMD): A Zoonotic Threat to Animal and Humans. Zoonosis Unique Sci Publ Faisalabad Pak 2023; 3: 637–650

[44] Grubman MJ, Baxt B. Foot-and-Mouth Disease. Clin Microbiol Rev 2004; 17: 465–493. doi:10.1128/CMR.17.2.465-493.2004

[45] RAOOF HS. Foot and Mouth Disease (FMD). Assiut Vet Med J 2024; 70: 1–8

[46] Khandke L, Yang C, Krylova K, et al. Preservative of choice for Prev (e) nar 13TM in a multi-dose formulation. Vaccine 2011; 29: 7144–7153

[47] Karthik K, Prabhu M. Bacterial diseases of goat and its preventive measures. In: Goat Science-Environment, Health and Economy. IntechOpen; 2021

[48] Songer JG. Clostridial enteric diseases of domestic animals. Clin Microbiol Rev 1996; 9: 216–234. doi:10.1128/CMR.9.2.216

[49] WHO. World Health Organization report: Manual on the management of the cold chain in vaccine storage and transport. 2008

[50] ISO. Quality management systems. 2020. Retrieved from https://www.iso.org. Accessed: 9 December 2025.

[51] Prasetya B, Restu Wahono D, Yopi, et al. Innovation opportunity and challenge of standardization in response to COVID-19 pandemic and the socio-economic impact: a case study in Indonesia. Standards 2022; 2: 66–82

[52] Thomas S, Abraham A, Rodríguez-Mallon A, et al. Challenges in Veterinary Vaccine Development. In: Thomas S, Hrsg. Vaccine Design. New York, NY: Springer US; 2022: 3–34

[53] Yadav MP, Singh RK, Malik YS. Emerging and Transboundary Animal Viral Diseases: Perspectives and Preparedness. In: Malik YS, Singh RK, Yadav MP, Hrsg. Emerging and Transboundary Animal Viruses. Singapore: Springer Singapore; 2020: 1–25

[54] Scott GY, Aborode AT, Adesola RO, et al. Enhancing Vaccine Quality and Accessibility: Strategies for Efficient Storage and Distribution in Resource-Constrained Environment. Outbreak Manag Response 2025; 1: 2443439. doi:10.1080/29947677.2024.2443439

[55] Abbas AO, Nassar FS, Al Ali AM. Challenges of ensuring sustainable poultry meat production and economic resilience under climate change for achieving sustainable food security. Res World Agric Econ 2025; 159–171