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Journal of Drug Delivery and Therapeutics

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Review Article

Experimental Animal Models for Influenza/Flu Virus Vaccine Development

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Article Info:

Article History:

Received 19 Nov 2023
Reviewed 6 Jan 2024
Accepted 23 Jan 2024
Published 15 Feb 2024

Cite this article as:

Gupta A, Satapathy T, Pradhan B, Sen K, Sahu S, Sahu AK, Bhardwaj SK, Khan MA, Experimental Animal Models for Influenza / Flu Virus Vaccine Development, Journal of Drug Delivery and Therapeutics. 2024; 14(2):192-204

DOI: http://dx.doi.org/10.22270/jddt.v14i2.6362

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Abstract

Objectives: The objective of this review is to explore the different animal models used in the development of Influenza / Flu Virus Vaccine as well as some important guidelines adhered to it.

Methods: Laboratory animal models are widely used in the preclinical evaluation of potential vaccines and antiviral compounds to investigate the safety and efficacy of the vaccine or compound in preventing or moderating infection, disease, or secondary transmission. Animal models must also represent humans in terms of similarity of clinical signs, histopathological changes, virus growth kinetics, or transmission. Common animal models for influenza virus vaccine testing include mice, ferrets, and non-human primates. These models help researchers assess vaccine efficacy, immune response, and potential side effects before advancing to human trials. Animal models play a crucial role in the development and testing of influenza vaccines, as they help researchers examine potential side effects and adverse reactions, evaluate the efficacy of vaccines, study the immune response elicited by vaccines, understand the pathogenesis of influenza virus infection, and predict the potential impact of emerging strains. They also help test various vaccine platforms and delivery methods, and compare the effectiveness of different vaccine formulations in animals to identify promising candidates for further human trials.

Results: We have discussed in detail the various animal models such as Chick embryo model for the development of influenza virus vaccine and mice, ferrets, guinea pig and non-human primates etc. used for the evaluation of safety and therapeutic effectiveness.

Conclusion: As we have discussed various animal models, their merits, demerits etc. for the evaluation of safety and effectiveness of influenza virus vaccine and expect that these will help for the young researchers to carry out their vaccine research using suitable animal models.

Keywords: influenza virus, vaccine development, mouse models, ferret model, guinea pig model, cotton rat model

Introduction:

The developed countries of the world are probably more aware of influenza (Flu) than of any other disease, except for the common cold. According to WHO (World Health Organization) report published on 2nd October 2023, there are around a billion cases of seasonal influenza annually, including 3–5 million cases of severe illness. It causes 290 000 to 650 000 respiratory deaths annually. The influenza virus consists of eight separate RNA (Ribo Nucleic Acid) segments of differing lengths enclosed by an inner layer of protein and an outer lipid bilayer as shown in the fig. no.1 below. Embedded in the bilayer are numerous projection that characterized the virus[1]. There are two types of projections: *hemagglutinin (H) spikes and neuraminidase (N) spikes.* There are eighteen different *hemagglutinin (H)* subtypes and eleven *neuraminidases (N)*. (H1 through H18 and N1 through N11, respectively)[2]. Laboratory animal models are widely used in the preclinical evaluation of potential vaccines and antiviral compounds, to investigate the safety of the vaccine or compound and its efficacy in preventing or moderating infection, disease or secondary transmission. Depending on the model species, read-outs can include clinical signs such as weight loss, lethargy, and pyrexia, or histopathological changes in or virus recovery from tissues such as nasopharynges or lung. Amelioration of these clinical, virological, or histopathological parameters in the presence of an investigational drug or vaccine suggests its antiviral efficacy in that animal model. The animal model must also represent humans, in terms of similarity of clinical signs, histopathological changes, virus growth kinetics, or transmission. Some animal models, like ferrets and guinea pigs, are naturally susceptible to infection by human influenza strains; others, like mice, require adaptation of the human virus to the species. Common animal models for influenza virus vaccine testing include mice, ferrets, and non-human primates. These models help researchers assess vaccine efficacy, immune response, and potential side effects before advancing to human trials[3].

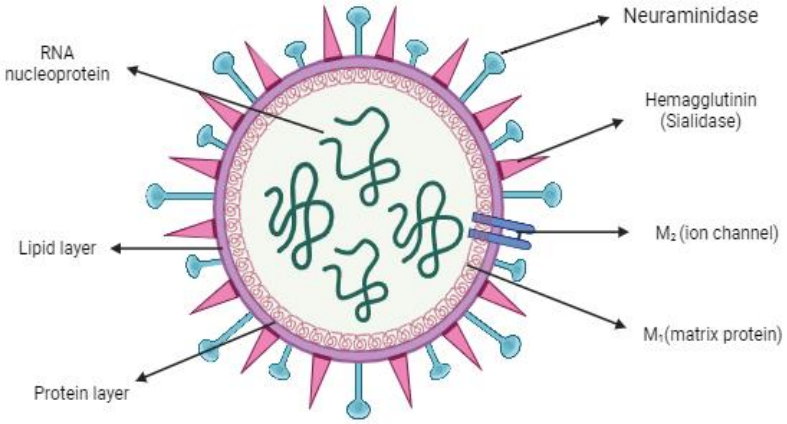

Figure 1: Diagrammatic representation of Influenza Virus

Epidemiology:

In India, the monsoon season (June to September) is often when influenza virus circulation peaks, with secondary peaks occurring in the winter (November to February). However, because different geographical regions have varied temperatures, the actual timing of the influenza season varies across the nation. Currently, the Government of India advises high-risk populations, such as healthcare professionals, expectant mothers, and those with long-term medical issues, to have an annual influenza vaccination. The government does not currently administer the influenza vaccine as part of its standard public health services[4, 5].

Pathophysiology:

Influenza is caused by influenza viruses, belong to the *Orthomyxoviridae* family. There are three main types. Influenza A and B viruses are responsible for seasonal flu outbreaks, while influenza C usually causes milder respiratory symptoms. Influenza viruses are primarily transmitted through respiratory droplets produced when an infected person talks, coughs, or sneezes. It also spread by touching a surface or object contaminated with the virus and then touching the mouth, nose, or possibly the eyes. Influenza viruses enter the body through the respiratory tract, specifically the nose and throat. The viral particles have surface glycoproteins called *hemagglutinin (HA) and neuraminidase(NA)* that play crucial roles in the infection process. *Hemagglutinin* binds to sialic acid receptors on the surface of host cells, facilitating viral entry. After cellular entry and once attached to the host cell, the virus is taken up by endocytosis. The viral RNA is then released into the host cell, where it undergoes transcription and replication as shown in fig. no. (2). The infected cell's machinery is hijacked to produce new viral particles. The body's immune system recognizes the presence of the virus and initiates an immune response. The innate immune system responds first, followed by the adaptive immune system. Influenza viruses can undergo antigenic changes, known as antigenic drift and antigenic shift, making it challenging for the immune system to recognize and respond effectively. In most cases, the immune system clears the infection, and the individual recovers. However, in some cases, the virus may persist or lead to chronic respiratory conditions. Understanding the pathophysiology of influenza is crucial for the development of effective prevention strategies, such as vaccination, and for the development of antiviral medications. Additionally, ongoing research helps improve our understanding of influenza virus evolution and the factors influencing its ability to cause seasonal epidemics and occasional pandemics[6, 7, 8].

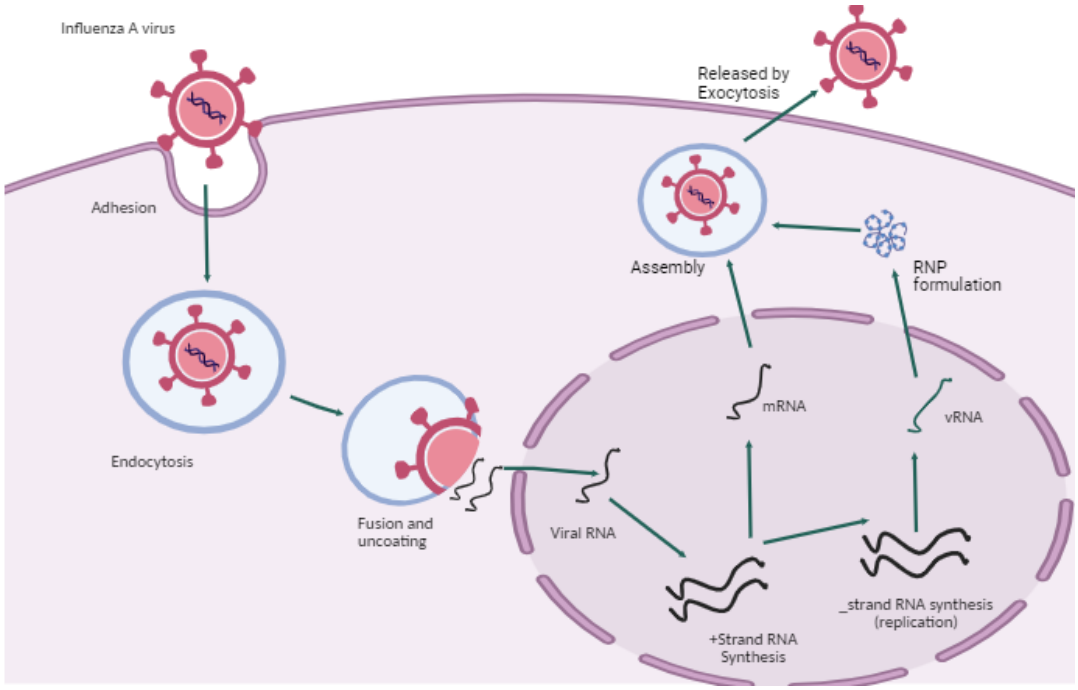

Figure 2: Diagrammatic representation of pathophysiology of influenza virus

Signs and Symptoms:

The common sign and symptoms of Influenza virus infection includes, Fever, Cough, Sore throat, discomfort and body pain, muscle and joint pain, Headache, Chill and sweat, Nasal congestion, Shortness of breath, Nausea and vomitingetc.[9, 10].

Need of animal model in influenza virus vaccine development:

Animal models play a crucial role in the development and testing of influenza virus vaccines. Researchers examine potential side effects and adverse reactions in animals to identify potential safety concerns. Animal models provide a platform to evaluate the efficacy of influenza vaccines in preventing infection and reducing the severity of symptoms. The immune response generated in animals can be studied to understand how well the vaccine induces protection against the virus. Animal models help researchers study the immune response elicited by influenza vaccines. [11, 12,13]

Table 1: The advantages and disadvantages of individual animal model in influenza research.

Animal Model	Advantages	Disadvantages
Mice	• The genome can be easily altered • handling and husbandry are simple • housing, upkeep, and reproduction are inexpensive • Reduced variability in inbred mouse strains • use in pathogenesis studies •Availability of virological and immunological reagents.	• There is a difference in the pathophysiology and appearance of the disease in mice, which makes them unsuitable for investigations on transmission • The immunological response in humans and mice differs.
Ferret	• Comparable human lung pathology and anatomy • Anatomical and physiological similarity to the human respiratory system • Suitable for pathogenesis, transmission, and vaccine efficacy investigations.	• Absence of immunological reagents unique to ferrets • Expensive handling and animal costs
Guinea pig	• Adaptable to different influenza strains naturally without prior adaption.	• Insufficient reagents •Minimal to nonexistent clinical symptoms.
Cotton rat	• Highly permissive model used for influenza virus • Low housing and experimental cost.	• Lower therapeutic index is difficult to determine.
Hamster	• Handling simplicity • Low maintenance costs • Influenza virus susceptibility • In research on vaccination efficacy and transmission.	• Absence of clinical symptoms.
Non-human primates	• Anatomical, physiological, and immunological feature similarities with humans in close genetic kinship • Susceptibility to several human and avian influenza virus ethical issues • Etiology and vaccination effectiveness trials • Comparable lung pathology to people.	• Expensive • Intricate husbandry requirements.
Swine	• Significant similarities between the human and avian influenza receptors in terms of anatomy, physiology, and genome sequences • High concentration of influenza receptors in the respiratory tract • Applied to research on transmission.	• Expensive • Challenging to handle.

Development of viral vaccine (influenza vaccine) using chick embryo model [14,15]:

With the advancement of time chicken embryo is used worldwide for development of candidate vaccine against influenza virus. The embryo generated eggs have been harvested and used for virus inoculation. The goal is to stimulate the immune cell that will be investigated further cells to fight against the viral infection.

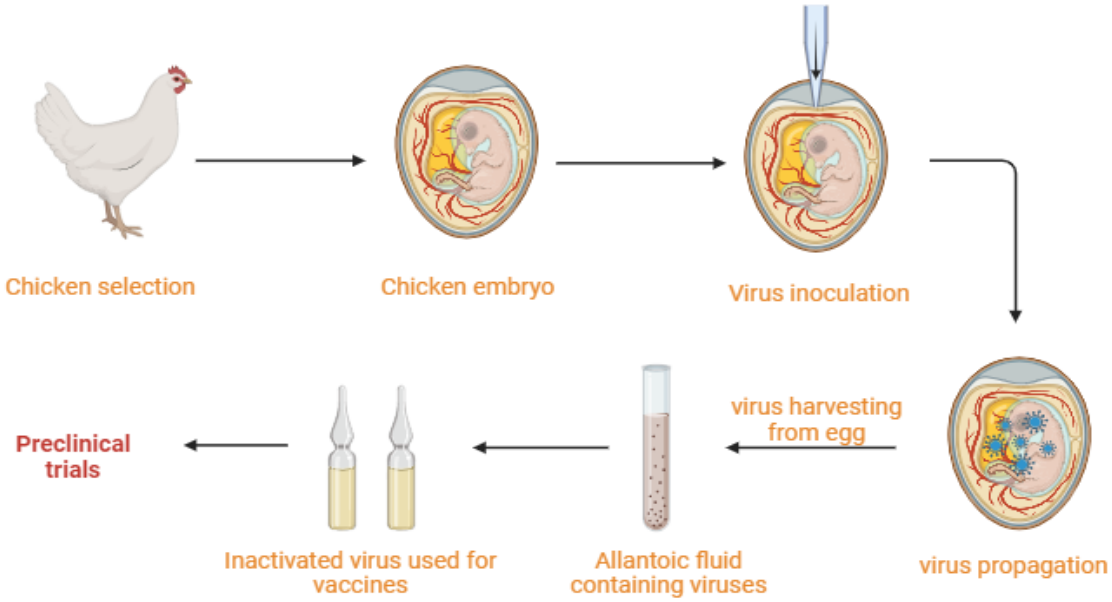

Figure 3: Diagrammatic representation of chicken model in influenza virus vaccine

Influenza viruses are typically inoculated into embryonated chicken eggs, which serve as a medium for viral replication. Once the viruses have replicated sufficiently, the allantoic fluid containing the virus is harvested from the eggs as shown in the above figure. Inactivated influenza viruses are often used to produce vaccines as they are free from pathogenicity. These viruses are treated to ensure they are no longer capable of causing disease but can still stimulate an immune response. The influenza vaccine is tested for safety, efficacy, and immunogenicity in preclinical studies using established animal models like mice, ferrets, or non-human primates etc.

Experimental animal models for Influenza virus vaccine

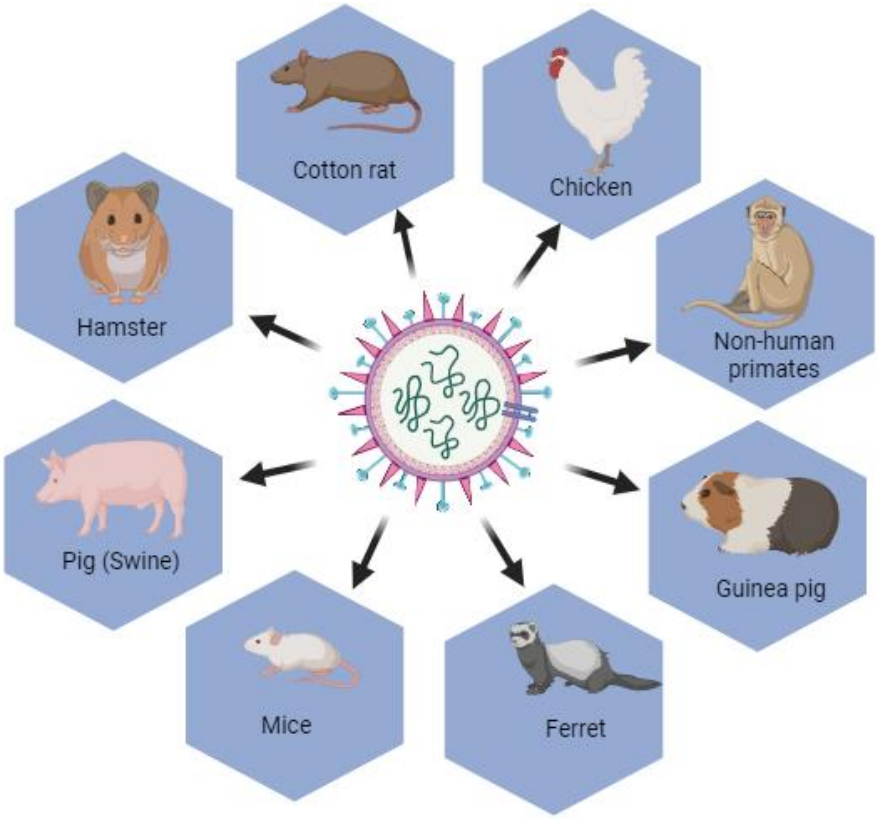

Figure 4: Diagrammatic representation of experimental animal models for influenza virus vaccine

1. Mice Model[16,17,18,19]**:**

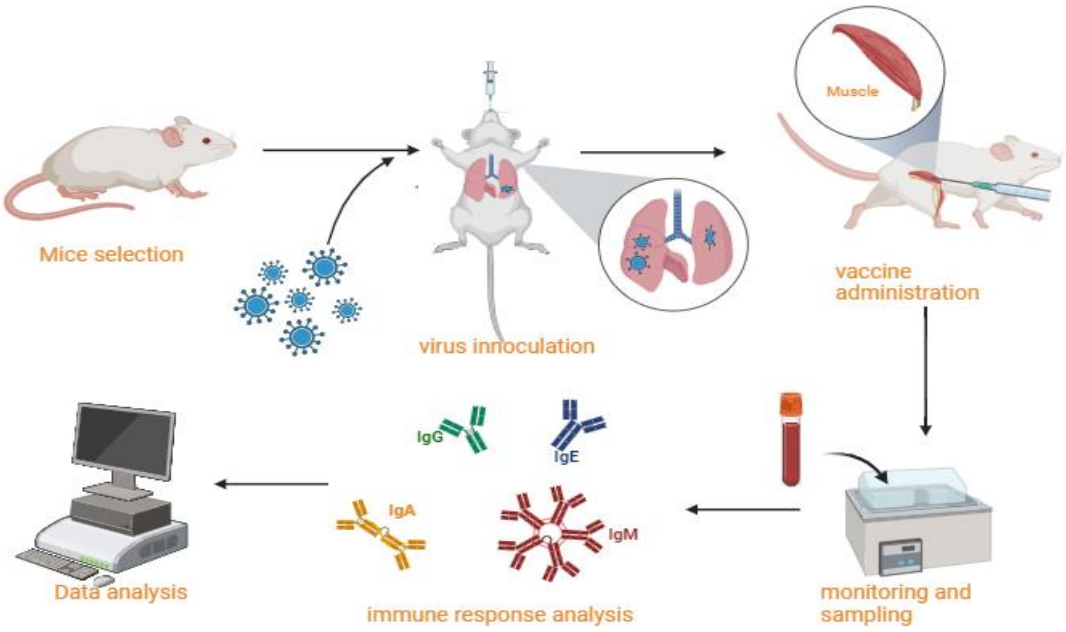

Figure 5: Diagrammatic representation of animal mice model in influenza virus vaccine

1.1. Mice selection

Choose a strain of mice that is suitable for the study. Common strains include BALB/c and C57BL/6.Consider factors like age, sex, and health status. Gender-balanced albino mice weighing between 12 and 28 g were housed in regular settings (26 ± 2 °C, 45 ± 2% relative humidity) and fed a standard pellet diet along with water.

1.2. Virus inoculation

Before conducting any experiments involving animals, researchers must obtain approval from an Institutional Animal Ethics Committee (IAEC). This ensures that the study follows ethical guidelines and minimizes the potential harm to animals. The mice are anesthetized, and a controlled amount of the virus is administered. The inoculation dose may vary depending on the strain and purpose of the study. Administer a controlled dose of the influenza virus to the mice, typically through intranasal or intramuscular injection. Before the mice were either exposed to the X31 virus or given an inoculation, they were put to sleep with 0.2 mL of Aveitin (2, 2, 2-tribromoethanol) by IP injection. The mice received a total inoculation of 0.1 mL with 106 TCID50/mL X31 viruses by intra nasal instillation at a rate of 0.05 mL/nare. The inoculation dose may vary depending on the strain and purpose of the study. After inoculation, mice are closely monitored for signs of illness, weight loss, and mortality followed by collect samples, such as blood, lung tissue, or nasal swabs, at various time points to analyze the progression of the infection and the host immune response.

1.3. Vaccine administration

Administration of the vaccine candidate to a group of mice, often through injection or other relevant methods. Control groups may receive a placebo or another vaccine for comparison. The appropriate route of administration is based on the research goals. Common routes include Subcutaneous (SC): Under the skin Intramuscular (IM)in to the muscle Intraperitoneal (IP) into the peritoneal cavity Intradermal (ID)into the skin.

1.4. Monitoring and sampling

The mice are regularly monitored for symptoms, weight loss, and mortality.Blood samples may be collected at specific time intervals to analyze immune responses, antibody production, and other relevant parameters. Changes in body weight can be indicative of overall health. Regular weighing helps identify potential issues, such as weight loss, which may be associated with illness or adverse reactions.

1.5. Immune response analysis

Various techniques are used to assess the effectiveness of vaccines and understand how the immune system responds. Samples are analyzed using techniques like ELISA (Enzyme-Linked Immunosorbent Assay) is commonly used to measure the presence and concentration of specific antibodies in the blood. Serum samples are analyzed to determine the levels of antibodies produced in response to vaccination. Flow cytometry is used for analyzing individual cells, immune cell populations, activation status, and cytokine production in a sample. Virus Neutralization Assays is used to assess the ability of antibodies to inhibit virus infectivity. These methods, among others, contribute to a comprehensive analysis of the immune response in mice following vaccination or experimental treatments.

1.6. Pathological examination

Mice are sacrificed after obtaining approval from Dissection Monitoring Committee (DMC). Tissues are then identified, isolated and preserved using appropriate fixatives (such as formalin) to prevent decay. Proper fixation is crucial for accurate pathological examination. Tissues and organs are examined for signs of infection or vaccine-induced protection. After harvesting the tissue the carcasses are disposed as per *CCSEA (Committee for the Control and Supervision of Experiments on Animals)*guideline to prevent spreading the infection. After completion of histopathological examination of tissue of interest, the data is analyzed to determine vaccine efficacy, immune response levels, and any adverse effects.

2. Ferrets model[20,21,22,23]:

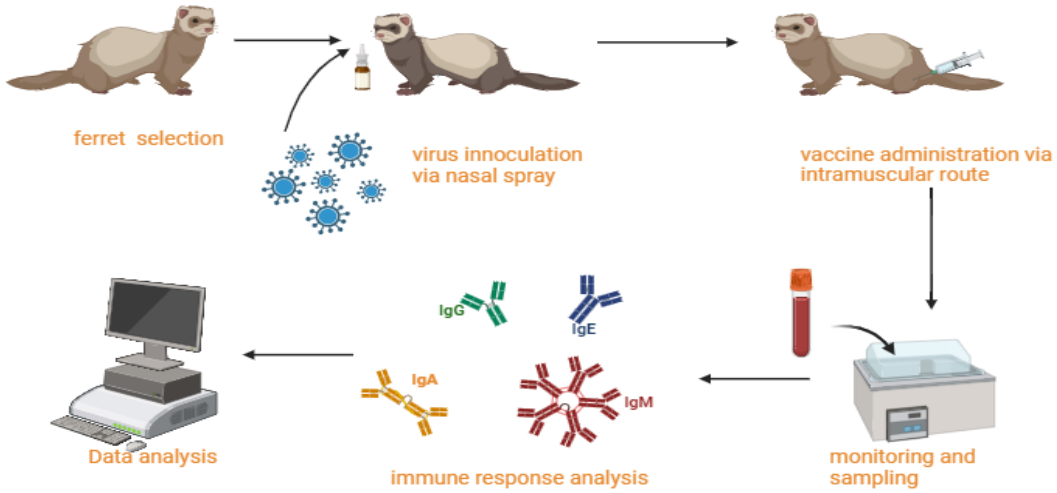

Figure 6: Diagrammatic representation of ferret model in influenza virus vaccine

2.1. Ferret selection and acclimatization

Healthy ferrets typically 6-12 months old are selected. The body weight of the ferret's are noted. Ferrets are allowed to acclimatize to the laboratory environment for a period of one week minimum before starting experiments. Sufficient amount of purified water and standard pallet diets should be provided to the ferrets and temperatures need to be maintained within 15 to 23°C. Other conditions should be as per CCSEA guideline.

2.2. Virus inoculation

Controlled dose of the influenza virus is administered via intranasal inoculation, to mimic natural infection. Ferrets can be infected with human influenza strains without the need for adaptation. Ferrets are often anesthetized before inoculation to minimize stress and facilitate handling. Common anesthetic agents used for ferrets include isoflurane or a combination of ketamine and medetomidine. The chosen influenza virus strain is then administered to the ferrets. Intranasal inoculation is a common method, as it mimics the natural route of infection in humans. The ferrets are then carefully monitored for signs of infection, such as changes in body temperature, weight loss, and respiratory symptoms. The viral titer, or the concentration of the virus, may also be measured at various time points.

2.3. Vaccine administration and clinical monitoring

Vaccine administration in ferrets for influenza virus typically follows a structured process to study the effectiveness of the vaccine in preventing infection or reducing the severity of the disease. The influenza candidate vaccine is administered to a group of ferrets, usually through intramuscular injection, subcutaneous injection, or intranasal administration. Control groups receiving placebos or alternative vaccines for comparison. The chosen method depends on the type of vaccine being tested. Ferrets may receive one or more booster doses of the vaccine at specified intervals and this assessment is used for the durability and efficacy of the immune response. Ferrets are closely monitored for any adverse reactions to the vaccine. Body temperature, weight, and others are health indicators. After the initial vaccination and any booster doses, ferrets may be challenged with a live influenza virus. This is done to simulate a natural infection and evaluate the protective efficacy of the vaccine.

2.4. Sampling

Collect respiratory samples (nasal washes, swabs) to measure viral load and shedding. Collect blood samples for serological analysis. When conducting influenza virus sampling in ferrets, various samples are collected to monitoring the course of infection, assess viral replication, and study the host immune response. Nasal washes involve instilling a saline solution into the ferret's nostrils, which is then aspirated back out to collect respiratory secretions. Nasal washes help quantify the amount of virus present in the upper respiratory tract and monitor changes in viral shedding over time. Throat swabs are taken by gently rubbing a cotton swab at the back of the ferret's throat. Throat swabs help detect the presence of the virus in the throat and monitor viral replication in the lower respiratory tract. Blood samples are typically collected through venipuncture and provide information about the host immune response, including the production of antibodies and other immune markers. Blood samples are often processed to obtain serum, which is the liquid component of blood after clotting. Serum contains antibodies, and its analysis helps assess the ferret's immune response to the influenza virus. Tissue biopsies provide insights into the distribution of the virus within different organs and the severity of tissue damage. Bronchoalveolar Lavage (BAL) fluid involves instilling and then withdrawing a saline solution from the lungs to collect the response of lower respiratory secretions. Fecal samples may be collected to assess the presence of the virus in the gastrointestinal tract.

2.5. Immune response analysis and pathological examination

Enzyme-linked Immunosorbent assay (ELISA) and other serological tests are commonly used to detect influenza virus-specific antibodies in serum. Serology helps assess the development of antibodies, including IgM (Immunoglobulin M) and IgG (Immunoglobulin G), against the influenza virus and measure the levels of various cytokines involved in the immune response. Cytokine profiling provides information about the host's inflammatory and immune response to the virus. Immune cells from blood or tissues are labeled with fluorescent markers and analyzed using flow cytometry to quantify different immune cell populations to response the influenza virus infection. After that the Pathological Examination takes place and tissues, including lungs, trachea, nasal passages, spleen, and others, are collected. Necropsy allows for a macroscopic examination of tissues and identification of lesions or abnormalities associated with influenza infection. Tissue samples are fixed, embedded in paraffin, sectioned, and stained for microscopic examination.

Histopathology helps identify cellular and tissue changes, including inflammation, necrosis, and other pathological alterations induced by influenza infection. Tissue sections are stained with specific antibodies to detect viral antigens or immune markers which is called Immunohistochemistry (IHC). IHC provides detailed information about the distribution of the virus within tissues and the localization of specific immune cells. Tissue samples are analyzed using Polymerase Chain Reaction (PCR) techniques to detect and quantify viral RNA or DNA (Deoxyribonucleic Acid) and viral load assessment helps quantify the extent of viral replication in different tissues.

3. Guinea pig model[24,25,26,27]:

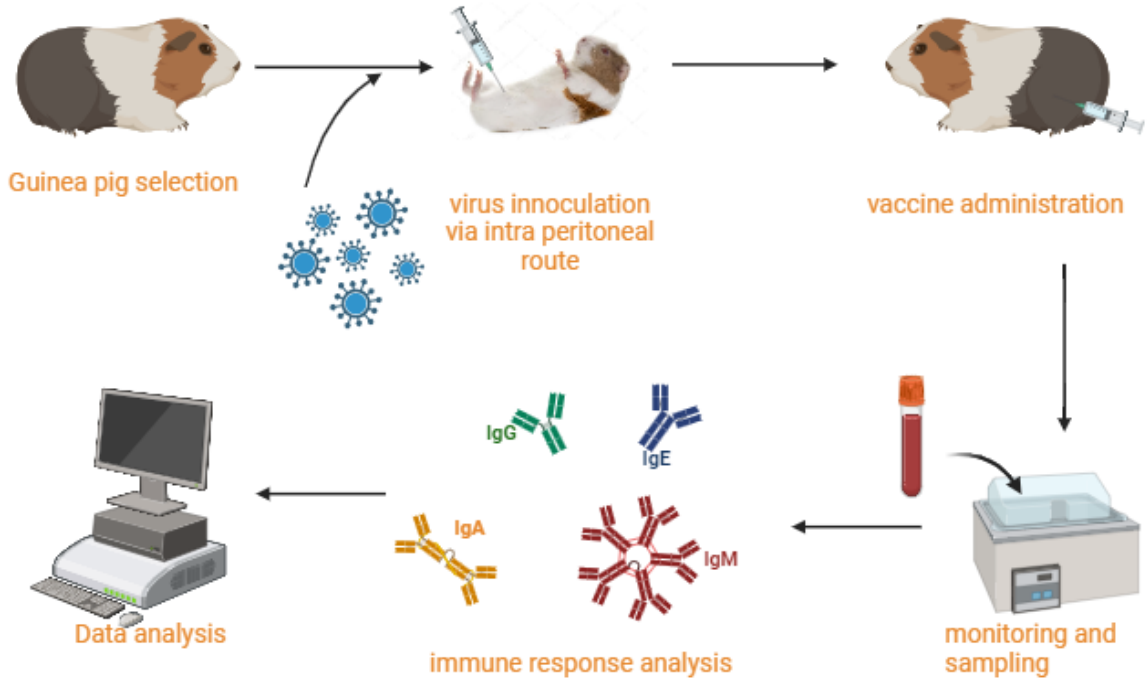

Figure 7: Diagrammatic representation of guinea pig model in influenza virus vaccine

3.1. Guinea pig selection and acclimatization

Healthy adult guinea pig of either gender is used for this study. Animals are procured from CCSEA approved breeder and transportation of animals should made lawfully as per CCSEA guideline. The selection of animals are done randomly and grouped into test or standard group as per protocol. Acclimatization is an important step in preparing guinea pigs for any experimental procedures, including vaccine administration. This process helps reduce stress and ensures that the animals are in optimal health for the study. Allow guinea pigs to acclimate to the laboratory environment for a period of seven days before starting experiments.

3.2. Virus inoculation

Controlled dose of the influenza virus is administered. After receiving an intraperitoneal inoculation of 3 × 104 plaque-forming units of the A/Wyoming/03/2003 virus, two guinea pigs (from appropriate group) were given a 5-week recovery period before receiving another injection of the same virus dose. Samples of nasal wash were taken 2, 3, 6, and 9 days after infection (dpi).

3.3. Vaccine administration

The influenza vaccine candidate is administered to a group of guinea pig typically through injection subcutaneously. The loose skin on the back of the neck or between the shoulder blades is a common site for subcutaneous injections. Intramuscular (IM) Some vaccines may be given intramuscularly, into the muscle. The hind leg muscles are commonly used for intramuscular injections.

3.4. Sampling and immunization

Respiratory samples (nasal washes, swabs) are collected to measure viral load and shedding. Blood samples are collected for serological analysis to assess antibody responses. Prior to vaccine-challenge investigations using comparable subunit antigens, the sensitivity of immune response to the HA (Hemagglutinin) antigen was first assessed using two doses of antigen. Four months after the last vaccination, the ELISA titers remained high and showed no signs of deterioration. HA-immunized animal's antiviral HI (Hemagglutination Inhibition) titers rose following their second, third, and fourth inoculations in both groups.

4. Cotton rat model[28,29,30,31]:

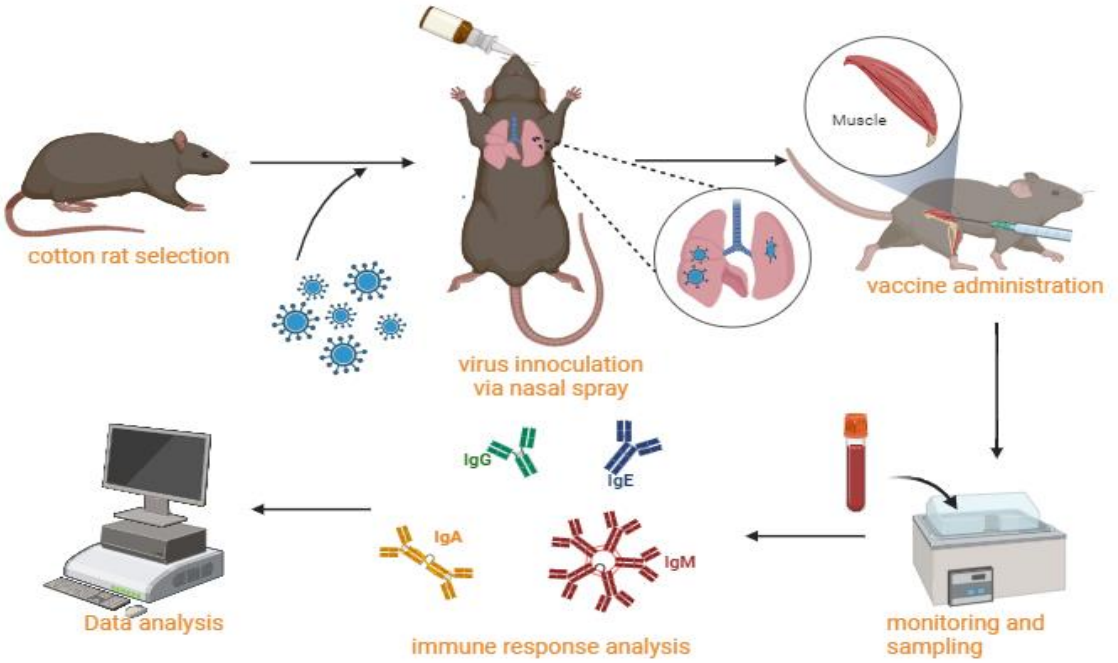

Figure 8: Diagrammatic representation of cotton rat model in influenza virus vaccine

4.1. Cotton rat selection and acclimatization

Natural isolates of influenza A and B viruses can infect cotton rats (Sigmodon hispidus or Sigmodon fulviventer) without the need for prior adaption. Cotton rats that are infected exhibit elevated respiratory rates, weight loss, and hypothermia. The assessment of potential vaccinations involves the measurement of viral titers in different tissues as well as clinical indicators. Although cotton rats are not as susceptible to infection as mice are, they are nonetheless not able to spread viruses among themselves. Choose healthy cotton rats for the study after considering practical factors such as age, sex, and health status. Newly procured animals are isolated and allowed for acclimatization for a period of one week prior to the initiation of experiment.

4.2. Virus inoculation

Influenza virus inoculation in cotton rats is conducted to understand aspects of the virus's pathogenesis, immune response, and to evaluate potential vaccines or antiviral treatments. The immune response to influenza infection is studied, including the production of antibodies and cellular immune responses and vaccine and antiviral testing, cotton rat may also be used to evaluate the efficacy of influenza vaccines and antiviral drugs. Virus Inoculation in cotton rat generally occurs via intranasal inoculation.

4.3. Vaccine administration

The influenza vaccine is prepared according to the experimental design. This may involve inactivating the virus or using live attenuated strains, depending on the goals of the study. Cotton rats are anesthetized before receiving the vaccine. This helps reduce stress and facilitates the administration process. Administration route of influenza virus in cotton rats are Subcutaneous or Intramuscular Injection depending on the experimental design.

4.4. Sampling and clinical monitoring

Experimental cotton rats need regularly monitoring for clinical signs such as weight loss, respiratory symptoms, and overall health. After vaccine administration animals are monitored for any signs of adverse reactions, immune responses for Inactivated Influenza Vaccines (IIVs), serum antibodies against hemagglutinin are thought to be correlates of vaccine-induced protection. Increased antibody levels brought on by vaccination reduce the chance of contracting an illness from strains that are antigenically identical to the strains of the same type or subtype that are contained in the vaccine and protection against subsequent viral challenges. Intra Nasal (IN) sample collection of nasal washes or swabs to measure viral shedding and assess mucosal immune responses, blood samples to analyze systemic immune responses and tissue samples, such as lung tissue, to examine the presence of the virus and assess the extent of infection.

5. Hamster model[32,33]:

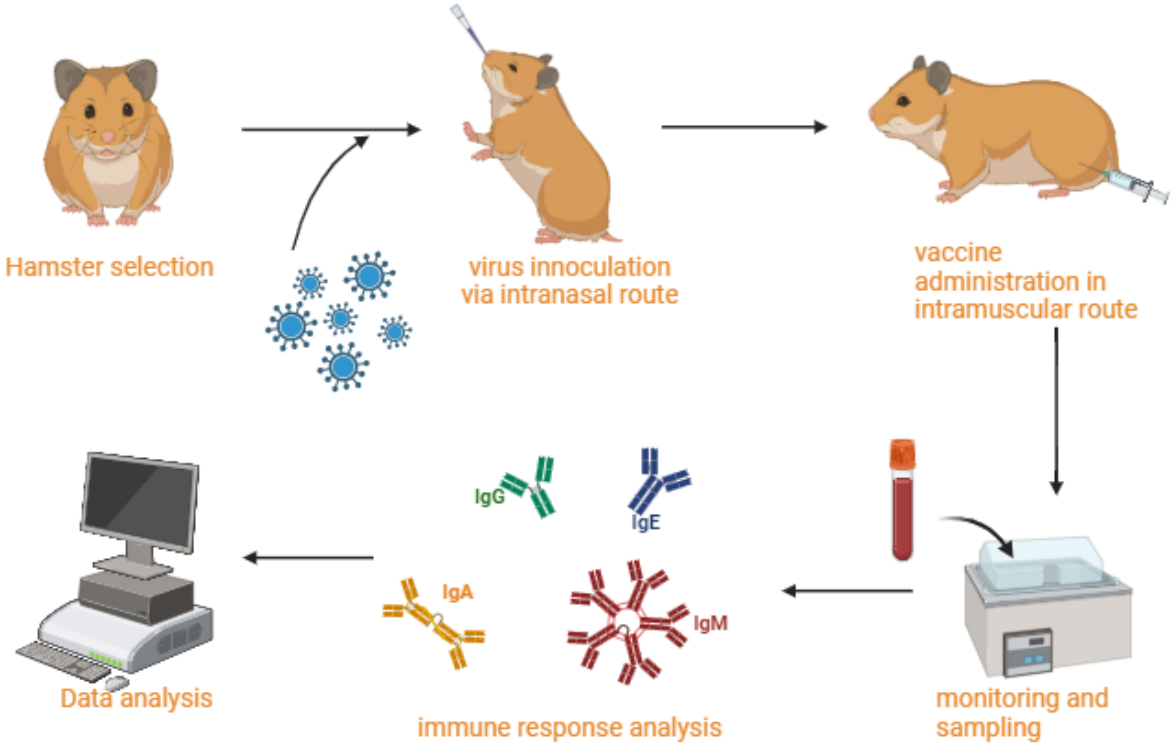

Figure 9: Diagrammatic representation of hamster model in influenza virus vaccine

5.1. Hamster selection and acclimatization

Healthy Syrian post weaning hamsters are selected for the study after considering factors such as age, sex, and health status. Hamsters are acquired from the CCSEA approved breeder randomly bred colony. The animals are weaned at 4 weeks of age, and when they weighed between 50 and 70 kg, they are employed for experiments at 6 to 12 weeks of age. Animals (hamsters) are allowed to acclimate to the laboratory environment for a period of seven days before starting experiments. Hamsters are nocturnal and can be easily stressed by loud noises. Provide a quiet and calm environment, especially during their active hours in the evening. Maintain a consistent feeding and playtime routine. Hamsters thrive on routine, and it helps them feel secure.

5.2. Virus inoculation

Hamsters are anesthetized using an appropriate anesthetic agent to minimize stress and facilitate handling during the procedure. Inoculation Influenza virus inoculation is typically performed via intranasal administration. The virus can be delivered in a liquid suspension, often in a small volume (e.g., 50-100 µL), using a micropipette. The inoculum may contain a specific influenza virus strain, and the virus titer should be determined to ensure consistent infection across experimental subjects.

5.3. Vaccine administration

The stable and sterile vaccine is administered to hamsters should be done with care and precision to ensure their well-being. Common injection sites include the scruff of the neck or the hind leg. Administer the influenza vaccine candidate to a group of hamsters, usually through intramuscular injection. Control groups receiving placebos or alternative vaccines for comparison. Regularly monitoring to be done on hamsters for clinical signs such as weight loss, respiratory symptoms, and overall health.

5.4. Blood sample collection and analysis

Blood samples may be analyzed to measure antibody levels, which can indicate the effectiveness of the vaccine in eliciting an immune response. Collection of respiratory samples (nasal washes, swabs) is done to measure viral load and shedding. Collection of blood sample is required for serological analysis to assess antibody responses. Control group hamsters are challenge vaccinated with a live influenza virus to assess vaccine efficacy.

5.5. Pathological examination

At termination of experiment, hamsters are sacrificed as per DMC guideline at designated time points to examine tissues and organs for signs of infection or vaccine-induced protection. The pathological examination involves the microscopic examination of tissues to identify any abnormalities, inflammation, or damage by analyzing tissues and organs from these animals to assess any potential effects or changes caused by the vaccine. The focus should be on specific organs relevant to the vaccine's target, such as the lungs, liver, or immune system organs. Immuno histochemical staining can be used to detect specific proteins in tissues.

6. Non-human primate's (NHPs) model[34,35,36,37,38]:

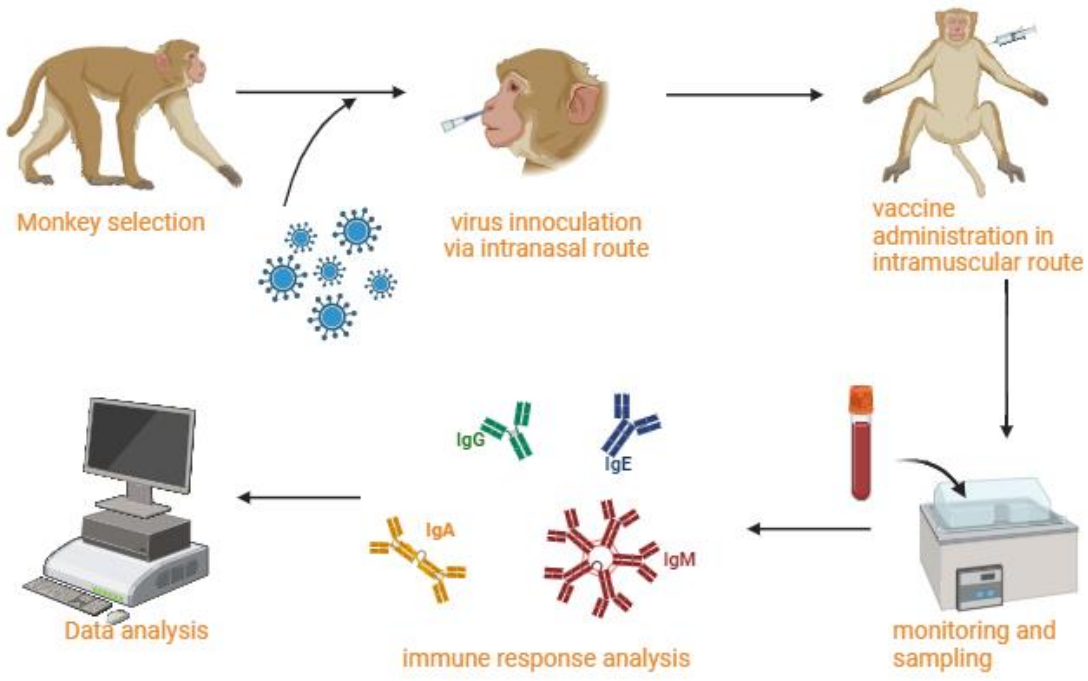

Figure 10: Diagrammatic representation of monkey model in influenza virus vaccine

6.1. NHPs selection and acclimatization

When selecting a monkey species as an animal model for influenza virus vaccine studies, we should consider various factors to ensure that the model is relevant, practical, and provides meaningful insights into human responses. Commonly used monkey species in influenza research include rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*Macaca fascicularis*), and African green monkeys (*Chlorocebus aethiops*). Appropriate species of NHPs such as macaques (e.g., rhesus or cynomolgus). Consider factors like age, sex, and health status is considered. Selected monkeys are allowed to adapt their new environment before the commencement of experiments. They should provide a comfortable and secure environment during transit and quarantine and health checks.

6.2. Virus inoculation

Influenza virus inoculation in Non-Human Primates (NHPs) is a common practice in scientific research to study the virus's pathogenesis, transmission, and to test the efficacy of vaccines and antiviral drugs. NHPs, such as macaques and marmosets, are used in influenza research because their respiratory and immune systems share similarities with humans. In Antiviral drug testing, assessing the effectiveness of antiviral drugs against influenza is intranasal inoculation. This method mimics natural infection through the respiratory route. Intra-tracheal inoculation is direct delivery of the virus into the trachea. Intramuscular inoculation is a method of inoculating the virus into the muscle that researcher monitors the NHPs for symptoms such as viral shedding, and immune responses. Regular collection of samples, such as nasal swabs, blood, and tissues, for analysis.

6.3. Vaccine administration

The influenza vaccine is prepared according to the study protocol and administered to anesthetize or sedate monkey to ensure a calm and cooperative state during vaccine administration. This is especially important for procedures like intramuscular injections. Common routes include intramuscular (IM) or subcutaneous (SC) injection, although other routes may be used depending on the study design.

6.4. Sampling and clinical monitoring

Clinical monitoring and sampling of monkeys during and after influenza vaccine administration are crucial components of research studies aimed at assessing the vaccine's efficacy, safety, and immune response. Regular observations are conducted for the first few hours after vaccination. Any changes in behavior, activity levels, appetite, and any signs of illness and vital signs, including heart rate, respiratory rate, temperature, and blood pressure, are required to be observed as needed. The blood samples are collected at designated time points post-vaccination to assess serum for influenza-specific antibody titers and other immunological markers. The nasal washes or swabs are collected to assess viral shedding and mucosal immune responses. This is particularly important for respiratory viruses like influenza. The tissue samples, such as lung tissues, are collected for further analysis.

7. The swine model39,40,41**:**

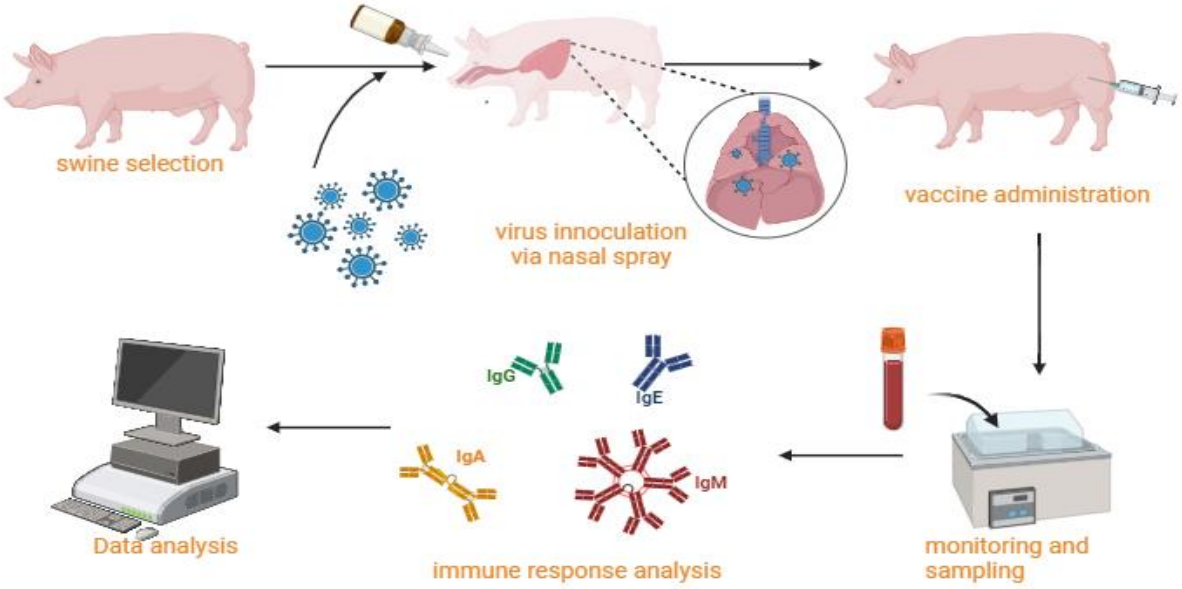

Figure 11: Diagrammatic representation of swine model in influenza virus vaccine

7.1. Swine selection and acclimatization

When selecting swine as an animal model for studying influenza virus, we should consider various factors to ensure the model is appropriate for their specific research objectives. Swine are share similarities with humans in terms of respiratory anatomy, immune responses, and susceptibility to certain influenza viruses, particularly those of the H1N1 subtype. Healthy pigs are chosen for the study, typically young and free from pre-existing health conditions. Swine influenza viruses, especially those of the H1N1 subtype, can infect both swine and humans. This zoonotic potential makes swine a valuable model for studying certain strains of influenza that can affect both species and Swine respiratory anatomy is similar to that of humans, with comparable airway structures and distribution. This similarity is important for studying the transmission and pathogenesis of influenza viruses. Before introducing swine to the research facility, a quarantine period of one week is implemented to monitor the animals for any signs of illness. Health checks, including screening for influenza and other diseases, are conducted to ensure that the swine are disease-free.

7.2. Virus inoculation

Before the inoculation, animals with pre-existing health issues may be excluded from the study. A specific influenza virus strain for inoculation is selected. The choice of strain may depend on the research objectives, including whether the goal is to study a specific subtype or strain. Swine may be anesthetized or restrained to facilitate the inoculation procedure and minimize stress. The route of inoculation is determined; common routes include intranasal, intra-tracheal, or intramuscular administration. A controlled dose of the influenza virus is administered. Pigs can be infected with both human and swine influenza viruses.

7.3. Vaccine administration

The influenza vaccine candidate is administered to a group of pigs, usually through intramuscular injection. Include control groups receiving placebos or alternative vaccines for comparison. Twenty domestic 5-week-old pigs that were devoid of both the swine IAV virus and its antibodies are selected. Following a week of acclimation, animals in one group are given the first dose of the commercial trivalent vaccination by intramuscular injection in the neck muscle (2 mL), in accordance with the manufacturer's recommendations.

7.4. Clinical monitoring and sampling

The pigs are monitored on regular basis for clinical signs such as temperature, respiratory symptoms, and overall health. Other signs of influenza infection, such as changes in behavior, respiratory symptoms, or changes in body temperature are also monitored. Collect samples, such as nasal swabs, blood, and tissue samples, at various time points to assess viral replication, immune response, and pathology.

Future Prospects:

With the Advancement of Science and Technology, *in-vitro* (outside the body) and *in silico* (computer-based) models may provide alternatives to some animal experiments. Organ-on-a-chip technology and human-on-a-chip models are also emerging as promising alternatives that mimic human physiology more closely. The development of genetically modified animals(e.g. nude mice) may allow for more precise modeling of human diseases and the testing of targeted therapies.

Conflict of interest: The authors declare no conflict of interest.

Acknowledgement: The authors are thankful to the management and principal, Columbia Institute of Pharmacy, Raipur (C.G.) for providing necessary facilities to complete this review article.

Funding: We do not receive any kind of funding from any source for this review article.

Author's contribution:

Ayushi Gupta and Bharti Pradhan: Preparation of content

Shailesh Sahu and Kalpana Sen: Diagram and figures

Trilochan Satapathy and Md. Altaf Khan: Grammar Correction and proof reading

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