MOHAMMED V UNIVERSITY IN RABAT
National School of Computer Science and Systems Analysis

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

Research Master in Bioinformatic and Complex Systems Modeling Applied to Healthcare

Subject:

Prediction of the optimal age for vaccination against H9N2 in chickens using mathematical modeling

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Technical report

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Chapter 1: Technical introduction

1. Project background

The background to this project is rooted in the poultry industry in Morocco, a sector that is essential for employment and food security in terms of animal proteins. Morocco is also a major exporter of poultry products to various markets, particularly in Africa.

However, in January 2016, the avian influenza virus subtype H9N2 was detected for the first time in poultry in Morocco, creating a worrying situation. In response to this threat, the National Sanitary and Security Food Office (ONSSA) authorized emergency vaccinations for all poultry farms. This proactive response aimed to contain the spread of the virus, protect poultry populations and minimize economic losses. This initiative demonstrated Morocco's commitment to animal health and the sustainability of the poultry industry.

2. Technical objectives and assumptions

The technical objectives of this project are as follows:

- To develop a mathematical model to determine the optimum time for vaccination against the H9N2 avian influenza virus in broilers.
- To implement this model using appropriate numerical methods and algorithms.
- Conduct experiments using relevant data to analyze the results and draw conclusions.

The underlying hypothesis is that this model will overcome the inhibitory effect of maternal antibodies, thereby improving vaccination efficacy. The critical period, including the first days of the chicks' lives and the period following the decrease in maternal antibodies, will be the focus of our research.

Chapter 2: Design and Architecture

In this chapter, we will explore the design and architecture of our modeling system for H9N2 avian influenza vaccination. This section will provide an understanding of how our project is structured and how its components interact to achieve our technical objectives.

1. System architecture and diagrams

The overall architecture of our system is based on a well-defined structure, illustrated in the diagram below:

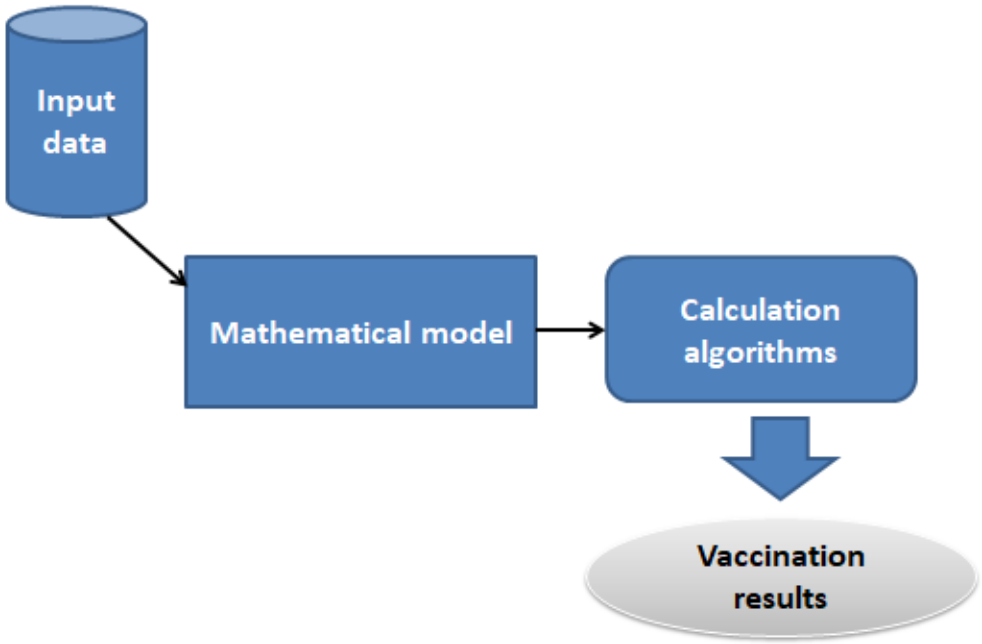

Figure 1:System Component Flow Diagram

This diagram highlights the main elements of our system, including the mathematical model, input data, calculation algorithms and vaccination results. Each component plays a crucial role in achieving our technical objectives.

2. Description of Technical Components

2.1. Mathematical model

The heart of our system lies in the mathematical model, which is based on a five-stage methodical approach, as described in the figure below:

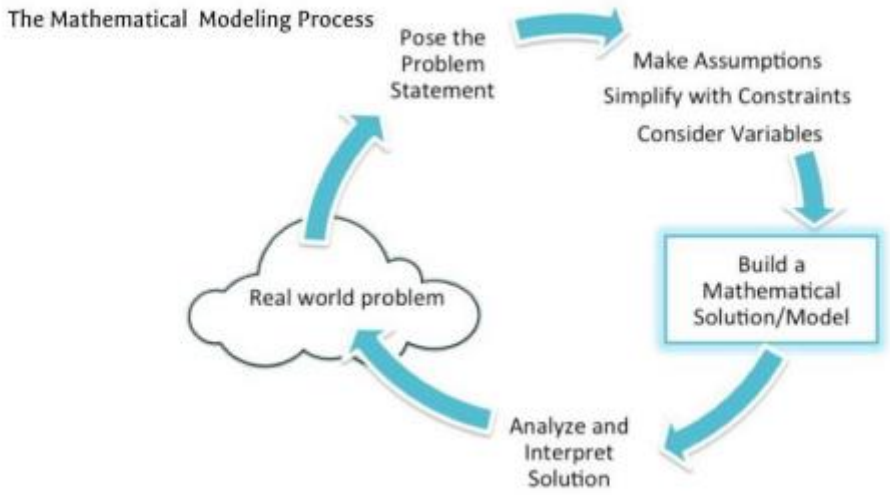

Figure 2: Mathematical modeling process

- **Step 1: Posing a Problem**

The first step is to clearly define the problem of vaccination against avian influenza H9N2 and to identify the specific research questions. This step focuses on our technical objectives.

- **Step 2: Formulate Hypotheses**

In the second step, we establish hypotheses based on existing knowledge and available information. These hypotheses identify the key factors influencing our model.

- **Step 3: Building a Model**

The third step involves formulating equations, relationships or mathematical rules that describe the vaccination system. These mathematical models simplify and abstract the interactions and behaviors of the system, making it easier to analyze and understand the problem.

- **Stage 4: Analyze and Revise the Model**

In this iterative phase, the model is progressively refined to ensure its relevance and validity. This ensures that the model is suitable for decision-making or for studying the phenomenon under study.

- **Stage 5: Validation and Use of the Model**

Finally, validation of the model is a crucial stage in ensuring its reliability and practical usefulness. This involves testing the model with empirical data and ensuring that it produces consistent and accurate results.

This mathematical model forms the basis of our modeling system and is essential for determining the optimum vaccination period.

2.2. Input data

Input data is an essential part of our system, as it feeds our mathematical model. This includes information on maternal antibody levels in broilers. This data is collected in collaboration with the IAV Hassan II-Rabat and is used to tailor the predictions of our model.

2.3. Algorithmes de Calcul

To solve the complex equations of the mathematical model, we implemented specific algorithms using the Python programming language. These algorithms were carefully chosen to ensure the accuracy of the results.

2.4. Vaccination results

The vaccination results, based on the predictions of our model, indicate the optimal period for vaccinating chicks against H9N2. These results provide valuable information for poultry health professionals, helping to improve vaccination practices and optimize poultry health.

By combining these technical components, our system offers a structured and rigorous approach to the challenge of H9N2 vaccination. The details provided in this section form the basis for our subsequent analysis and experimentation, as described in the following chapters.

Chapter 3: Mathematical modeling

In this chapter, we present our own personalized mathematical modeling approach to determining the optimal age for vaccination against the H9N2 virus.

1. Data description and preprocessing

1.1. Datasets description

Our datasets contain measurements of maternal antibodies in broiler chicks reared in isolation at the Avian Pathology Unit of Hassan II-Rabat Agronomic and Veterinary Institute (IAV). Maternal anti-H9N2 antibody titers of the both dataset were measured using the hemaggglutination inhibition assay (HIA) according to the procedure β (constant antigen and serum serially diluted) according to the OIE manual (OIE, 2019). The standard Moroccan strain H9N2 antigen was obtained from the Laboratory of the Pharmacy and Veterinary Inputs Division, as well as ELISA.Two commercial ELISA kits were used to determine the titer of antibodies of maternal origin against the LPAI H9N2 virus: ID Screen® Influenza H9 Indirect for the detection of antibodies directed against the H9 haemagglutinin of the Influenza A virus, and ID Screen® Influenza A Nucleoprotein Indirect for the specific detection and assay of antibodies directed against the nucleoprotein of the Influenza A virus. These ELISA kits were run according to the manufacturer's recommended protocol using an automated microplate reader (ELx800, BIO-TEK Instruments Inc, Winooski, VT).

1.1.1. Variation of Maternal anti-H9N2 antibody without vaccination:

The dataset contains measurements of maternally derived antibodies in 20 individuals.
Blood samples were taken by both kits on day one (D1) and every 7 days thereafter until the individuals reached 42 days of age.

First of all this is the data that I received:

Jours		1	7	14	21	28	35	42
individu 1	FLUNPS	11 649	5 006	4 595	3 174	2 775	896	375
	FLUH9S	18 811	12 107	7 452	2 640	1 038	237	155
individu 2	FLUNPS	11 642	4 328	4 228	3 073	884	424	299
	FLUH9S	18 393	7 319	6 082	2 612	503	202	83
individu 3	FLUNPS	11 543	4 319	2 519	2 985	855	291	258
	FLUH9S	17 874	6 013	5 022	2 538	486	153	81
individu 4	FLUNPS	11 529	3 475	2 391	2 575	565	287	202
	FLUH9S	17 827	4 448	4 897	1 949	465	95	47
individu 5	FLUNPS	11 487	3 463	2 080	1 137	514	273	139
	FLUH9S	17 687	4 419	3 638	1 871	349	86	47
individu 6	FLUNPS	11 465	2 906	1 832	1 134	489	229	131
	FLUH9S	17 656	4 290	3 539	1 730	278	75	42
individu 7	FLUNPS	11 371	2 289	1 484	1 111	399	151	131
	FLUH9S	16 549	3 865	3 147	1 633	271	58	28

Figure 3:First data of Variation of Maternal anti-H9N2 antibody without vaccination

This data was not ready for pre-processing, for that I set up another one with the same inputs as shown in the figure 12:

individu	jour	MDA FLUNPS	MDA FLUH9S
individu 1	1	11 649	18 811
individu 1	7	5 006	12 107
individu 1	14	5 006	7 452
individu 1	21	3 174	2 640
individu 1	28	2 775	1 038
individu 1	35	896	237
individu 1	42	375	155
individu 2	1	11 642	18 393
individu 2	7	4 328	7 319
individu 2	14	4 228	6 082
individu 2	21	3 073	2 612
individu 2	28	884	503
individu 2	35	424	202
individu 2	42	299	83
individu 3	1	11 543	17 874
individu 3	7	4 319	6 013
individu 3	14	2 519	5 022
individu 3	21	2 985	2 538
individu 3	28	855	486
individu 3	35	291	153
individu 3	42	258	81
individu 4	1	11 529	17 827
individu 4	7	3 475	4 448

Figure 4:Data of Variation of Maternal anti-H9N2 antibody without vaccination

The data has 4 features with 140 rows:

- **Individu** : identifies each individual and is represented by a unique number, it has 20 modes individu1;….; individu20 .
- **Jour**: quantitative variable that represent the age of blood sampling contains the 7 days of age (1, 7, 14, 21 28, 35, 42) for each chick.
- **MDA FLUNPS**: MDA measured using FLUNPS kit for each individual on a given day. It is a quantitative variable of min 23 and max 11649.
- **MDA FLUH9S**: MDA measured using FLUH9S kit for each individual on a given day. It is a quantitative variable of min 1 and max 18811.

1.1.2. Variation of Maternal anti-H9N2 antibody with vaccination:

The second set of data concerns the variation in antibodies each week by trying vaccination at different times: at day 1, between 5 and 7 days, and finally at 14 days. Blood samples were taken on day 1 and every 7 days thereafter until individuals reached 35 days of age. Measurements were taken for each batch using both kits.

So the data that I received has 3 sheets; every sheet concern an age of vaccination.

LOT	Vaccin	1	7	14	21	28	35
		FLUNPS	FLUNPS	FLUNPS	FLUNPS	FLUNPS	FLUNPS
1	C	9355	5115	1378	312	878	1146
2	C	13910	12537	6723	909	335	596
3	C	7568	6697			797	951
4	C	7701	4840	1332	530	431	1269
5	C	9791	6683	2295	519	326	245
		FLUH9S	FLUH9S	FLUH9S	FLUH9S	FLUH9S	FLUH9S
			11106	6148	920	95	
			12534		774	399	
		14505	13908			190	555
		12235	7566	1418	322	149	
			11830	7344	2988	489	127

Figure 5:First vaccine data

The figure 13 shows there is some empty cells especially for FLUH9S kit, also some observations doesn't represent an immune response. Since we are interested on the immune response of the vaccination, I create a data that contains only the rows when an immune response is observed for FLUNPS kit (the number of MDA increase in taking account the age of vaccination plus the latency because we are working with an inactivated vaccine).

lot	jour_vaccin	jour	MDA
1	5j_7j	1	9355
1	5j_7j	7	5115
1	5j_7j	14	1378
1	5j_7j	21	312
1	5j_7j	28	878
1	5j_7j	35	1146
2	5j_7j	1	13910
2	5j_7j	7	12537
2	5j_7j	14	6723
2	5j_7j	21	909
2	5j_7j	28	335
2	5j_7j	35	596
3	5j_7j	1	9791
3	5j_7j	7	6683
3	5j_7j	14	2295
3	5j_7j	21	519
3	5j_7j	28	326
3	5j_7j	35	245
4	1	1	7772
4	1	7	3142
4	1	14	608
4	1	21	923
4	1	28	1114
4	1	35	870

Figure 6: Vaccine data

The new data contains 46 rows with 4 columns:

- **Lot**: identifies each batch and is represented by a unique number. Lots are identified as "lot 1", "lot 2", …, "lot7"
- **Jour_vacin** : the time of vaccination ,there's : 1 , 14 and 5j_7j, which means that the vaccination took place between 5 and 7 days.
- **MDA**: MDA measured using FLUNPS kit for each batch on a given day;
- **Jour**: represent the age of blood sampling contains the 6 days of age (1, 7, 14, 21 28, 35) for each batch. It is a quantitative variable of min 245 and max 13910

1.2. Datasets preprocessing:

1.2.1. Variation of Maternal anti-H9N2 antibody without vaccination:

The first step was to find the missing values and their percentages for each column. In our case, after running the query, we find that there are no missing values.

```
la dataframe selectionnée 4 colones 140 lignes.
il y a 0 colonne qui ont des valeurs manquantes.
   Zero Values  Missing Values  % of Total Values
```

Figure 7:Missing values results

After that we plot a graph showing the geometric mean titers of maternal anti-H9N2 antibodies at different ages by the two ELISA test kits.

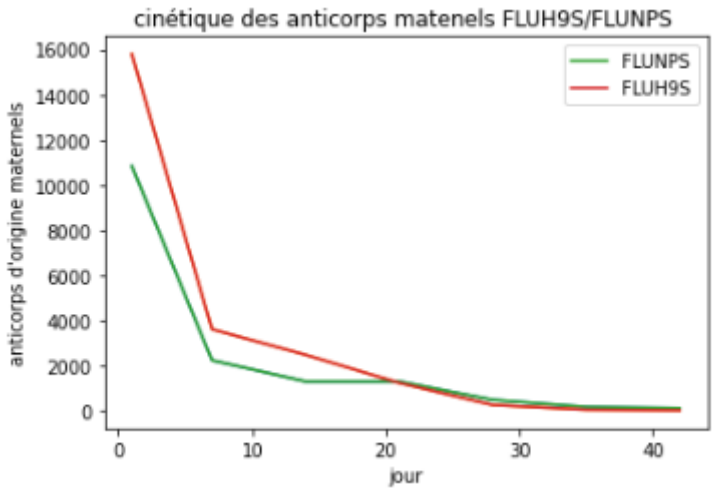

Figure 8: Maternal antibody cenitics FLUNPS/FLUH9S

Anti-H9N2 antibodies showed a progressive decrease in titer and was almost exhausted at D28.

1.2.2. Variation of Maternal anti-H9N2 antibody with vaccination:

In this dataset we found that there are no missing values. As mentioned before, we are interested here on the immunity response, for that we did an operation consists of replacing the values in the 'MDA' column with zero if the value in the 'jour' column is less than the sum of the value in the 'jour_vaccin' column and 14 ,because we will still have antibodies degradation. Otherwise, the value in the 'MDA' column remains unchanged.

	lot	jour_vaccin	jour	MDA
0	1	7	1	0
1	1	7	7	0
2	1	7	14	0
3	1	7	21	312
4	1	7	28	878
5	1	7	35	1146
6	2	7	1	0
7	2	7	7	0
8	2	7	14	0
9	2	7	21	909
10	2	7	28	335
11	2	7	35	596

Figure 9:Preprocessed data_vaccine

Then we plot the graph of the mean titer of maternal anti-H9N2 antibodies at different ages to represent the immunity response

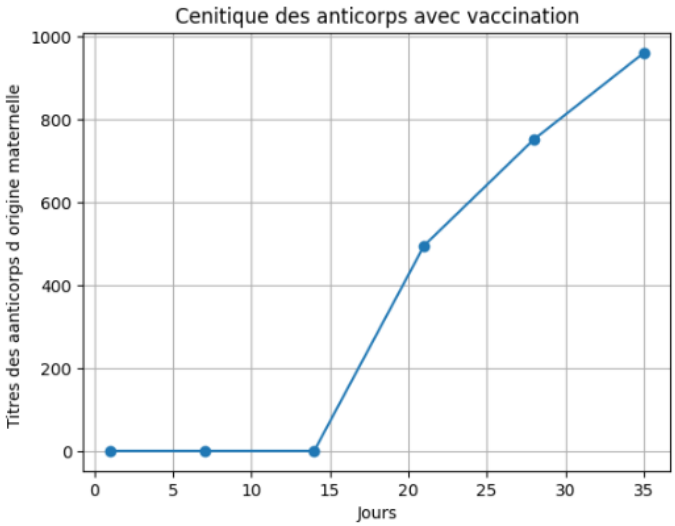

Figure 10: Graphical Representation of Mean Titers of Maternal Anti-H9N2 Antibodies at Different Ages: Immune Response Analysis

Anti-H9N2 antibodies showed a progressive increase in titer with time after the 14 day.

2. Mathematical modeling of the problem

2.1. Antibody degradation modeling

The figure 18 shows that the decrease of maternal antibodies follows a decreasing exponential curve. It can therefore be represented using the equation:

$$A(t) = A0 * e^{-kt} + C$$

With:

- **A(t)** : the level of maternal antibodies at a given time t
- **A0** : the initial level of maternal antibodies
- **k**: the degradation rate of maternal antibodies.
- **C**: constant

In fact, an adjustment must be made in order to determine the values of the parameters A0, k, and C of the above equation. The adjustment entails comparing experimental data or

information provided with equation predictions, then estimating the parameter values that minimize the difference between the two.

The method of least squares, is frequently employed for curve adjustment. This method involves minimizing the sum of the squares of the differences between the observed values and the values predicted by the equation.

We check the quality of the fit by calculating the coefficient of determination R^2, which indicates the proportion of variation in the data that is explained by the equation.

2.2. Immune response modeling

The level of remaining maternal antibodies is a very important factor, which can affect the poultry to produce their own immune response to the vaccine. In some cases, the level of maternal antibodies may be high and may inhibit the immune response to the vaccine, while in other cases the level of maternal antibodies may be low response to the vaccine, while in other cases the maternal antibody level may be low and may allow a stronger immune response.

The figure shows that the increase of maternal antibodies follows a logarithmic curve. It can therefore be represented using the equation:

$$Y(t)= Y0 * \log (k1* t)+C1$$

With :

- **Y(t)**: the level of maternal antibodies at a given time t (the time elapsed since vaccination in days)
- **Y0**: the initial level of antibodies as soon as we start to see a response
- **k1**: the growth rate of antibodies
- **C1**: constant

2.3. Model 1:

This model will be based on the cut_off defined by a unit of activity in a serodiagnostic test which makes it possible to identify whether an animal is probably infected by a given pathogen by measuring the quantity of antibodies present in its blood. If antibody activity exceeds the threshold, the animal is considered positive, indicating a low probability of infection, while activity below the threshold classifies it as negative, indicating a high

probability infection[40]. In our case, ELISA tests using the FLUH9S kit showed a cut-off of 732, while the FLUNPS kit showed a cut-off of 668.

The goal of this model is to find the age where all the chicks arrive to the cut-off so we can apply the vaccination.

Based on the equation of antibody degradation, we determine the equation of the time required:

$$t = -\frac{1}{k} * log\left(\frac{cut_{off} - C}{A0}\right)$$

with :

- **k** : the degradation rate of maternal antibodies
- **Cut_off** : The threshold of the ELISA kit
- **A0** : the number of antibodies on the first day
- **C** : constant

2.4. Model 2

This second model will be an optimization problem, requiring the modeling of both antibody degradation and the immune response to be taken into account, it will be implemented only for FUNPS kit. Before we start, we need to talk about latency, which is very important in the study of an inactivated vaccine, and which represents the time needed before an immune response occurs, in our case estimated at 14 days.

❖ Antibody degradation modeling

The breakdown of antibodies will be influenced by the age of vaccination, since a latency period is required before the immune response develops. When we vaccinate at a certain age, we have to wait until the end of this latency period to observe the development of the immune response. However, during this period, antibodies can break down. So the age of vaccination plays a crucial role in the dynamics of antibody degradation and the immune response.

$$A(t, \text{age\_vaccine}) = \begin{cases} A0 * e^{-kt} + C & if \quad t \leq \text{age\_vaccine} + \text{latency} \\ 0 & if \quad t > \text{age\_vaccine} + \text{latency} \end{cases}$$

The parameters A_o, k and C are the same calculated by fitting the exponential function for the FLUNPS kit. (Figure 21 and 22)

❖ Immune response modeling

In this section, we will simulate the dynamics of the immune response, taking into account factors such as the age of vaccination and the latency period.

Note that z = age_vaccine +latency

$$Y(t, age\_vaccine) = \begin{cases} Y0 * log(k1 * (t - z)) + C1 & if \ t > z \\ 0 & if \ t \leq z \end{cases}$$

The parameters Y0, k1 and C1 are the same calculated by fitting the logarithmic function for the FLUNPS kit. (Figure23)

When modeling the immune response to the vaccine, the expression "t - z" is used to calculate the time elapsed since the end of the latent period. This allows the immune response to be taken into account only after the end of this crucial period, whereas before it, the immune response is considered to be zero.

❖ Objective function

This function represents the whole process of the degradation of maternal antibodies and the immune response to the vaccine. It combines these two components to estimate the total quantity of antibodies at a given age.

The first component, **A_residual,** corresponds to the quantity of maternal antibodies remaining at the age given. It is calculated using the function **A (age, age_vaccine),** which models the decrease in maternal antibodies over time.

$$A\_residual = A(age, age\_vaccine)$$

The second component, **Y_vaccine** represents the immune response to the vaccine. It is calculated using the function **Y (age, age_vaccine**), which models the growth of antibodies in response to vaccination.

$$Y\_vaccine = Y(age, age\_vaccine)$$

So the objective function:

$$Objective\ function(age, age\_vaccine) = A\_residual + Y\_vaccine$$

❖ Function f

A function **f (age_vaccine)** has been defined which plays a central role in the search for the optimal vaccination age. It uses the **objective_function** to calculate the total amount of antibodies at 35 days of age, taking into account both the degradation of maternal antibodies and the immune response to the vaccine. It should be noted that the age of 35 days was deliberately chosen to allow the observation of a significant immune response. Given that the maximum age of vaccination is 21 days, taking into account a latency of 14 days, the maximum age at which we can assess the immune response is 34 days. By choosing an age of 35 days, we ensure that we have enough days after the latency period to obtain a complete picture of the immune response.

By inverting the sign of the **function_objective** in the function **f(age_vaccine),** we transform the problem into a minimization problem, where we seek to find the vaccination age that gives the smallest negative value, which corresponds to the largest total quantity of antibodies.

❖ Constraints

Two constraints were defined. The first constraint ensures that the vaccination age does not exceed 21 days. It is defined by the function constraint (age) = age - 21. This ensures that we take into account the practical limits of the vaccination age.

The second constraint, called constraint1, is a non-linear constraint that applies to the function f (age_vaccine). This constraint limits the value of f (age_vaccine) to not be less than a given value. After experimental tests we have chosen the value 500 as a limit value which means that the totality of the maternal antibodies must not be less than 500.

❖ Optimal age

The **age_optimal** function aims to determine the optimal vaccination age by maximizing the total amount of antibodies at 35 days of age, while respecting the defined constraints. It uses the minimize optimization method predefined to find the optimal value.

The bounds of the vaccination age are defined between 14 and 21 days, which correspond to the period during which vaccination can be carried out.

2.5. Model 3

In this modeling approach, we consider the dynamics of chicks' immunity to the H9N2 virus as a Continuous-Time Markov Chain (CTMC). CTMCs are commonly used to describe processes that evolve over continuous time and involve transitions between different states. The purpose of using CTMC for Modeling Chick Immunity

- ***Continuous Immunity Changes***: Chickens' immunity levels change continuously over time. It's not a discrete process that occurs at fixed intervals. CTMC is well-suited to capture such continuous changes.
- ***Transitions with Time Delays***: The transitions between immunity states involve time delays. For example, it takes time for antibodies to degrade, for a vaccination response to develop, or for an infection to manifest. CTMC allows us to model these transitions with precision

2.5.1. States in the Model

In this model, we define five distinct states that represent the possible conditions of chicks in response to the H9N2 virus. Each state is characterized by an initial letter:

- ***State M (Maternally Protected):*** At birth, chicks benefit from maternal immunity against the virus. This means they have sufficiently high antibody levels for protection.
- ***State S (Susceptible):*** In this state, chicks are susceptible to infection by the virus, having not yet developed immunity. They are at risk of infection.
- ***State V (Vaccinated):*** When a chick is vaccinated against the H9N2 virus, it enters this state. Vaccination aims to stimulate a specific immune response.
- ***State I (Infected):*** This state represents the situation where a chick is infected with the H9N2 virus. It is currently carrying the infection.
- ***State PV (Protected by Vaccination):*** If a chick has been successfully vaccinated and has developed a sufficient immune response to protect against the virus, it enters this state.

2.5.2. Possible Transitions between States

Understanding the interactions between states is crucial for comprehending the dynamics of infection and vaccination in chicks. Here are the possible transitions:

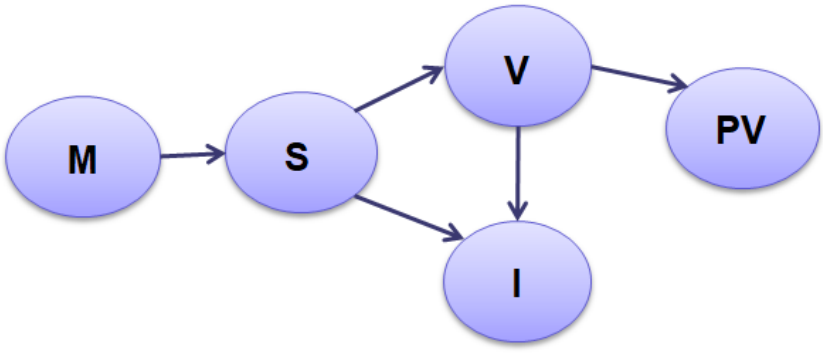

Figure 11:markov chain diagram in continuous time

- **Transition from State M to State S**: Chicks are born in State M, but over time, maternal antibody levels decrease. When these levels reach a critical threshold, chicks become susceptible to infection and transition to State S.
- **Transition from State S to State I**: If a chick in State S is exposed to the virus and its antibody levels do not provide protection, it can become infected and transition to State I.
- **Transition from State V to State I**: If a chick is vaccinated while in State S and its antibody levels are already above a critical threshold, it may still become infected for a period after vaccination. This delay can vary based on the antibody level at the time of vaccination.
- **Transition from State S to State V**: Chicks can be vaccinated at a specific age, moving them from State S to State V. The timing of vaccination depends on when the vaccination is administered.
- **Transition from State V to State PV:** To move from State V to State PV, a chick must develop a sufficient immune response after vaccination, reaching a critical antibody level that protects against the virus.

2.5.3. Hypotheses for State Transitions

For each transition, we have formulated specific hypotheses regarding antibody levels, time intervals, and critical thresholds. These hypotheses are based on empirical data and knowledge of chick immunity.

- ***Transition from State M to State S:***

Antibody Level Hypothesis: We assume that maternal antibody levels gradually decrease after chick hatching. We have established a critical threshold, corresponding to 20% of initial antibody levels, below which chicks are considered susceptible to infection.

Degradation Modeling: To model maternal antibody degradation, we use an exponential curve representing the gradual decrease in antibody levels over time. This curve is based on empirical data.

- ***Transition from State S to State I:***

Antibody Level Hypothesis: If a chick in State S is exposed to the virus and its antibody levels do not provide protection, it can become infected.

- ***Transition from State S to State I after Vaccination:***

Antibody Level and Delay Hypothesis: After vaccination, there is a period during which chicks are not yet fully protected because the immune response takes time to develop. This delay varies based on the antibody levels at the time of vaccination. If antibody levels are already high, the delay may be short, but if they are lower, the delay may be longer.

- ***Transition from State S to State V:***

Vaccination Timing Hypothesis: Chicks can be vaccinated at different ages, depending on the operator's choice. This transition is based on the decision to vaccinate chicks at a specific age.

- ***Transition from State V to State PV:***

Antibody Level after Vaccination Hypothesis: To transition from State V to State PV, a chick must develop a sufficient immune response after vaccination. This immune response is reflected in antibody levels reaching a critical threshold, typically determined by ELISA kits.

- ***Transition from State V to State I:***

Antibody Level and Delay Hypothesis: If a chick's antibody level at the time of vaccination is insufficient to protect against infection, it can still become infected after vaccination. The delay before the immune response becomes effective can vary depending on the antibody levels at the time of vaccination. If levels are low, the delay may be longer.

2.5.4. Generating Transition Matrices for Different Vaccination Ages

We are constructing transition matrices to model the dynamics of the H9N2 virus infection and vaccination process for various vaccination ages, ranging from 14 to 20 days. The purpose of these matrices is to capture the probabilities of transitioning between different states within the Markov chain model. Here's a breakdown of the steps and matrices involved:

- **Generative Matrix** : This matrix represents the rates of transition between different states. The values in this matrix depend on the duration of stay in each state (e.g., "S," "V") before transitioning. You have calculated these durations based on vaccination age choices. For example, for "M" (Maternal Protection), the transition rate to "S" (Susceptible) is calculated as 1 divided by the duration of stay in "M" before transitioning to "S."
- **Transition Matrix**: This matrix defines the probabilities of transitioning from one state to another. The values in this matrix are derived from the generative matrix. For instance, the transition probability from "S" to "V" is based on the duration of stay in "S" before vaccination, and it accounts for the probability of infection (0.5) during that time.
- **Absorbing States**: In your model, the "I" (Infected) and "PV" (Protected by Vaccination) states are absorbing states, meaning once a chick enters these states, they cannot transition out. This is represented in the transition matrix by having diagonal elements equal to 1 for these states.
- **Transient States:** The remaining states, "M," "S," and "V," are transient states, which means they are not absorbing. These states allow for transitions to other states.

- **Q Matrix:** The Q matrix represents the submatrix of transient states in the transition matrix. It is essential for further calculations related to the Markov chain.
- **Identity Matrix:** This is an identity matrix used in the calculation of the fundamental matrix.
- **Fundamental Matrix:** The fundamental matrix helps determine expected time spent in transient states before absorption occurs.
- **R Matrix:** The R matrix is used to calculate the probabilities of absorption from transient states into absorbing states.
- **Matrix of Absorption Probabilities:** This matrix provides the probabilities of eventually absorbing into each absorbing state (either "I" or "PV") from transient states.

These matrices are crucial for analyzing the dynamics of the Markov chain and understanding how different vaccination ages impact the probabilities of infection, protection, and duration of susceptibility in your model.

2.5.5. Problem of this model

Certainly, let's discuss the issue with your Markov chain model. The problem you've identified in your generative matrix arises from the assumption that a chick can transition directly from state "S" (Susceptible) to state "V" (Vaccinated) without the possibility of transitioning to state "I" (Infected) first. This assumption might not accurately represent the real-world dynamics of infection and vaccination.

The issue lies in the generative matrix, which models the rates of transition between states. In your current model, you have transition rates directly from "S" to "V" and from "S" to "I." This means that, theoretically, a chick in the "S" state could become vaccinated without ever becoming infected. However, in practice, there might be cases where a chick becomes infected before vaccination or where vaccination follows infection.

Similarly, the model allowed direct transitions from the "V" state to either "PV" (Protected by Vaccination) or "I" (Infected). In reality, vaccination doesn't guarantee instant protection, and there could be instances where chicks transition to the "I" state post-vaccination.

These limitations arise from the oversimplification of transition pathways and do not fully capture the complexities of chick immunity, vaccination, and infection dynamics. Addressing these issues is crucial to ensure that the model aligns more closely with real-world scenarios in poultry health management.

Chapter 4: Implementation and Programming

This chapter focuses on the implementation and programming of our three models for studying the dynamics of infection and vaccination in chicks exposed to the H9N2 virus. The main objective of this section is to present in detail the models we developed, highlighting the technological choices we made throughout the implementation process.

1. Model 1

1.1. Technological Choices

For the implementation of Model 1, we made the following technological choices:

- **Python**: Python was selected as the primary programming language for its versatility, extensive scientific libraries, and readability. These qualities make it well-suited for modeling biological processes.
- **Pandas:** We employed the Pandas library for data manipulation and management. Pandas' data structures and functions facilitated efficient data handling.
- **SciPy:** a scientific computing library, was utilized for curve fitting. It provided the tools necessary to fit an exponential curve to the observed antibody data.
- **Matplotlib:** was chosen for data visualization. This library allowed us to create informative plots for visualizing the fitted curves and results.

These technological choices were deliberate, aimed at ensuring flexibility, robust data handling capabilities, and the ability to accurately model antibody dynamics.

1.2. Code Organization

The source code for Model 1 is structured methodically to ensure clarity, maintainability, and effective execution. Here is an in-depth breakdown of its organization:

- Data Import:

We initiate the implementation by importing essential data from an Excel file named 'data_antibodies.xlsx' using the Pandas library.

The dataset comprises daily observations of antibody levels, which serve as the foundation for modeling.

- Exponential Curve Fitting:

Model 1 hinges on the assumption that maternal antibodies degrade exponentially over time. To facilitate this, we define an exponential function using the numpy library. This function is represented as exp_func(x, a, b, c), where:

- x represents time,
- a is a scaling factor,
- b denotes the decay rate, and
- c signifies the baseline antibody level.

The core functionality here is the fitting of this exponential curve to the observed antibody data. This is accomplished using the curve_fit function from the SciPy library.

Fitted parameters, including the decay rate (k1) and baseline antibody level (c1), are extracted for further analysis.

- Threshold Calculation:

An essential aspect of Model 1 is determining the time required for antibody levels to reach a critical threshold. This threshold, such as 668 for the FLUNPS kit, is integral in determining the optimal age for vaccination.

The code defines a function time_to_threshold(A_0, k1, c1, threshold) that calculates the time (t) necessary for the antibody level to reach the specified threshold. This function is based on the exponential decay model and extracts the decay rate (k1) and baseline level (c1) calculated earlier.

- Data Analysis:

After fitting the exponential curve and calculating the time to reach the threshold for each data point, we perform a data analysis.

We create an empty list, age_to_threshold, to store the calculated times for each observation.

For each observation in the dataset, the code calculates and appends the time to reach the threshold to this list.

We further compute summary statistics such as the mean and maximum time required to reach the threshold. These statistics provide critical insights into the dynamics of maternal antibody degradation, aiding in the determination of optimal vaccination timing.

By organizing the code in this structured manner, we ensure that each component of Model 1 is clearly defined and modular. This organization enhances code readability and maintainability, making it easier to extend and adapt the model as needed.

2. Model 2

2.1. Technological Choices

- Python: Python was selected as the primary programming language for its versatility, extensive scientific libraries, and readability. These qualities make it well-suited for modeling biological processes.

- Pandas :The library was utilized for efficient data manipulation and analysis. It provides a powerful DataFrame structure to handle tabular data, facilitating various data operations such as filtering, grouping, and transformation.

- Matplotlib: The library was employed for data visualization and plot generation. It offers a wide range of plotting functions and customization options to create clear and informative visual representations of the data.

- NumPy :a fundamental library for numerical computations in Python, was utilized for efficient handling of multi-dimensional arrays and mathematical operations. It provided the necessary tools for numerical computations and data manipulation.

- SciPy :The library was used for its extensive collection of scientific computing functions.
  Specifically, the following functionalities were employed:

 ***scipy.optimize.curve_fit*:** This function was employed for performing curve fitting using the least squares method. It facilitated finding the optimal parameters of a mathematical model that best fits the observed data.

 scipy.optimize.minimize: The minimize function from SciPy was used to solve the optimization problem and determine the optimal value. It allowed for efficient iterative optimization by considering the specified constraints.

 scipy.optimize.NonlinearConstraint: The NonlinearConstraint class from the scipy.optimize module was used to incorporate a nonlinear constraint into the optimization process. It provided the ability to impose additional conditions on the optimization problem.

- scikit-learn (sklearn.metrics): The scikit-learn library's sklearn.metrics submodule was employed to evaluate the performance of the fitted models. Specifically, the r2_score function was utilized to calculate the coefficient of determination R2, which assesses the goodness of fit of the models to the observed data.

2.2. Code Organization

- **Import Statements:** These lines import necessary Python libraries and modules.

pandas is used for data manipulation.

numpy provides numerical and mathematical functions.

curve_fit from scipy.optimize is used for curve fitting.

minimize from scipy.optimize is used for optimization.

randint from the random module generates random integers

- **Random Initialization:** These lines introduce randomness into the model. The latence variable is initialized to 14, but if a random number x is less than 4, latence is adjusted by a small random value between -1 and 1. This represents variability in chick responses.
- **Modeling Maternal Antibody Decay:** This function models the decay of maternal antibodies over time. It takes parameters t (time), age_vaccin (age at vaccination), A_0 (initial antibody level), k (decay rate), and c (constant term). It calculates the antibody level at time t using an exponential decay model. If t is greater than or equal to z, it returns 0, indicating that maternal antibodies are no longer effective.
- **Modeling Vaccination Response:** This function models the response to vaccination. It takes similar parameters as the previous function and calculates the response using a logarithmic function or another function specified by log_func. The response only starts after a certain time (z) has passed since vaccination.
- **Objective Function:** This function combines the maternal antibody decay and vaccination response to form an objective value for optimization. It calculates and returns the sum of these two components.
- **Objective Function for Optimization:** This function represents the objective to be optimized. It takes the age of vaccination as a parameter and calculates the corresponding objective value based on the components defined earlier.
- **Constraint:** This line defines a constraint on the optimization problem. It ensures that the objective value is less than or equal to -50 (an arbitrary value chosen for the constraint).
- **Optimization Function**: This function finds the optimal age for vaccination by minimizing the objective function. It considers the constraint and returns the optimal age.

- **Optimization Result:** These lines call the optimization function to find the optimal age for vaccination, round the result, and print the objective value for this optimal age.

This code structure combines modeling, optimization, and constraint handling to determine the best age for vaccination in a poultry context. It takes into account both maternal antibody decay and the vaccination response to make informed decisions.

Chapter 5 : Results and Discussion

In this chapter, we present the results of our study and engage in a detailed discussion of the findings. We aim to provide a comprehensive analysis of the data and insights gained from our modeling efforts.

1. Antibody degradation modeling

An adjustment was made in order to determine the values of the parameters A0,k, and C of the above equation. The adjustment entails comparing experimental data or information provided with equation predictions, then estimating the parameter values that minimize the difference between the two. The method of least squares, is frequently employed for curve adjustment. This method involves minimizing the sum of the squares of the differences between the observed values and the values predicted by the equation.

We obtain:

```
A_0 = 19235.811483978792 , k = 0.23817466954379318 , c = 553.838193677667
<ipython-input-59-0c7f894857f1>:6: RuntimeWarning:

overflow encountered in exp
```

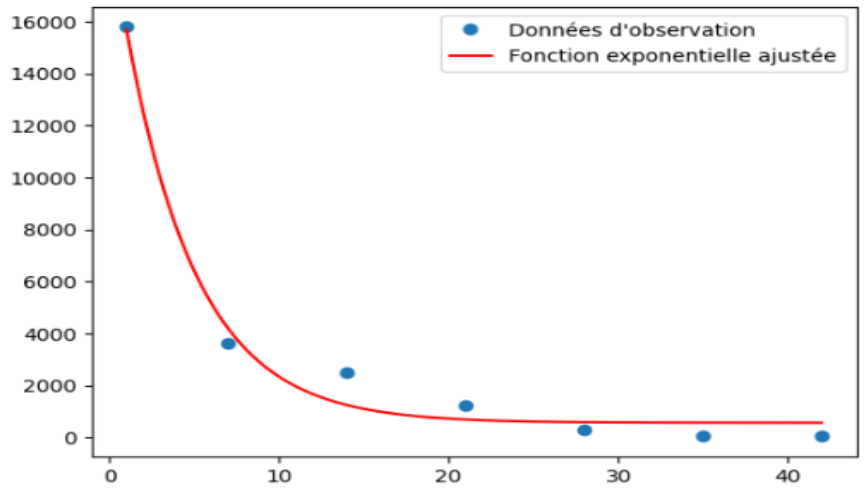

Figure 12: Adjustment for FLUH9S kit

For FLUH9S kit, k is 0.238, this would mean that 23.8% of the remaining maternal antibodies decompose per week.

```
A_0 = 13625.854108515605 , k = 0.28738947672854714 , c = 595.9708455273094
<ipython-input-11-4ba5bb19189c>:5: RuntimeWarning: overflow encountered in exp
  return a * np.exp(-b * x) + c
```

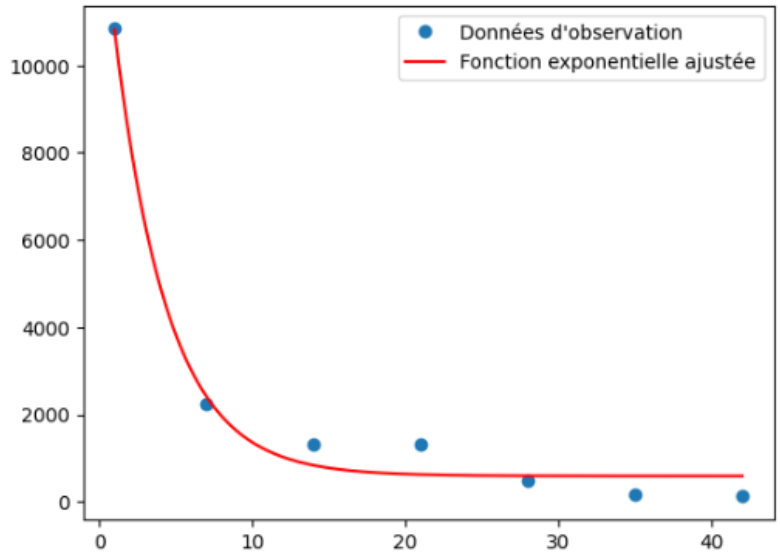

Figure 13:Adjustment for FLUNPS kit

For FLUNPS kit, k is 0.287 this mean that 28.7% of the remaining maternal antibodies decompose per week.

We check the quality of the fit by calculating the coefficient of determination R^2, which indicates the proportion of variation in the data that is explained by the equation.

The R^2 values obtained are 0.985 for FLUH9S and 0.987 for FLUNPS. It is high for both kits and close to 1, indicating that the equation is a good fit to the observed data.

2. Immune response modeling

The method of least squares is applied to make a fit and to determine the values of the parameters:

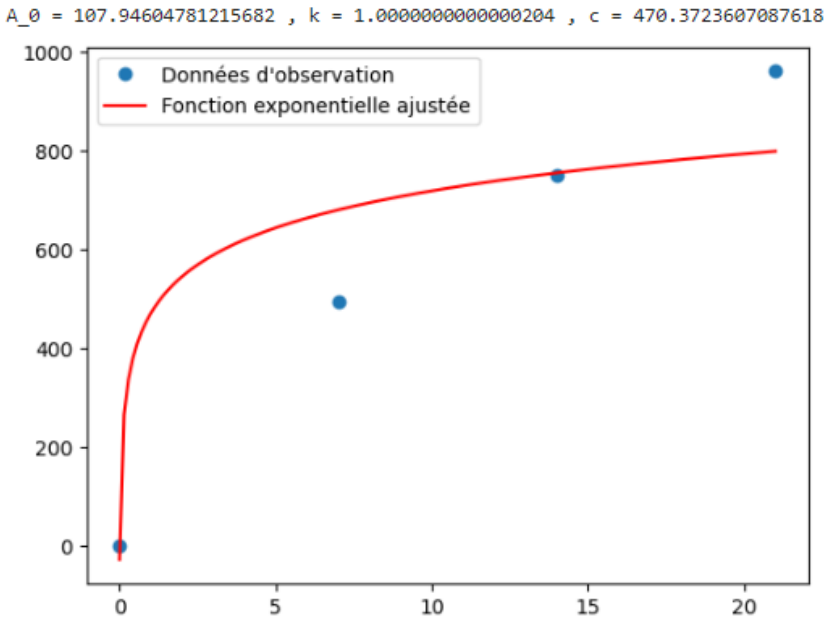

Figure 14:Adjustment for data vaccine

Note: this adjustment concern only the FLUNPS kit

After that the R^2 was calculated **.** The R^2value obtained is 0.881, it is high indicating that the equation is a good fit to the observed data.

3. Model 1 and Model 2

Using the model based on the cutoff defined by each serological test kit, we find the following results:

	MDAFLUH9S_j1	ageMDAFLUH9S
0	18811	19.563393
1	18393	19.469044
2	17874	19.348867
3	17827	19.337813
4	17687	19.304710
5	17656	19.297344
6	16549	19.025485
7	16174	18.929250
8	16159	18.925355
9	16058	18.899030
10	15655	18.792315
11	15232	18.677307
12	15186	18.664608
13	15178	18.662396
14	15106	18.642432
15	14873	18.577166
16	14853	18.571517
17	12472	17.837955
18	12107	17.713247
19	11911	17.644720

Figure 15:model1 results for FLUH9S kit

For the FLUH9S kit, we observed that the chicks reached the cutoff of 732 at ages ranging from 19.563393 days for the first chick to 17.644720 days for the last chick. These results indicate that the chicks took on average around 1.92 days to develop sufficient antibody activity to be classified as positive according to the FLUH9S kit criteria.

	MDAFLUNS_j1	ageMDAFLUNPS
0	11649	17.696907
1	11642	17.694815
2	11543	17.665099
3	11529	17.660876
4	11487	17.648177
5	11465	17.641506
6	11371	17.612860
7	11339	17.603054
8	11191	17.557338
9	11141	17.541757
10	11026	17.505653
11	10882	17.459910
12	10796	17.432302
13	10789	17.430045
14	10721	17.408045
15	10396	17.300931
16	9719	17.066620
17	9657	17.044352
18	9603	17.024840
19	8778	16.712278

Figure 16:Model1 results for FLUH9S kit

On the other hand, for the FLUNPS kit, with a cutoff of 668, the chicks reached this threshold at different ages. The first chick reached the cutoff at 17.696907 days, while the last chick reached it at 16.712278 days. These results suggest that the chicks required on average approximately 0.98 days less to reach the level of antibody activity required to be considered positive according to the FLUNPS kit criteria compared to the FLUH9S kit.

In resume, the average number of days taken to reach the cutoff of 668 was approximately 17.44 days, with a maximum of 17.70 days. This indicates that most chicks reached the required level of antibody activity at the same time.

For the FLUH9S kit, the average number of days taken to reach the cutoff of 732 was approximately 18.79 days, with a maximum of 19.56 days. This suggests that the chicks generally took slightly longer to reach the required level of antibody activity compared to the FLUNPS kit.

This difference in time to cutoff can be attributed to a number of factors, including the composition and specific efficacy of the test kits, as well as individual variation in the immune response of the chicks.

The second model, which used a different approach, gave the result that 21 days is the optimal age for vaccination, with an antibody level of 596,554 units.

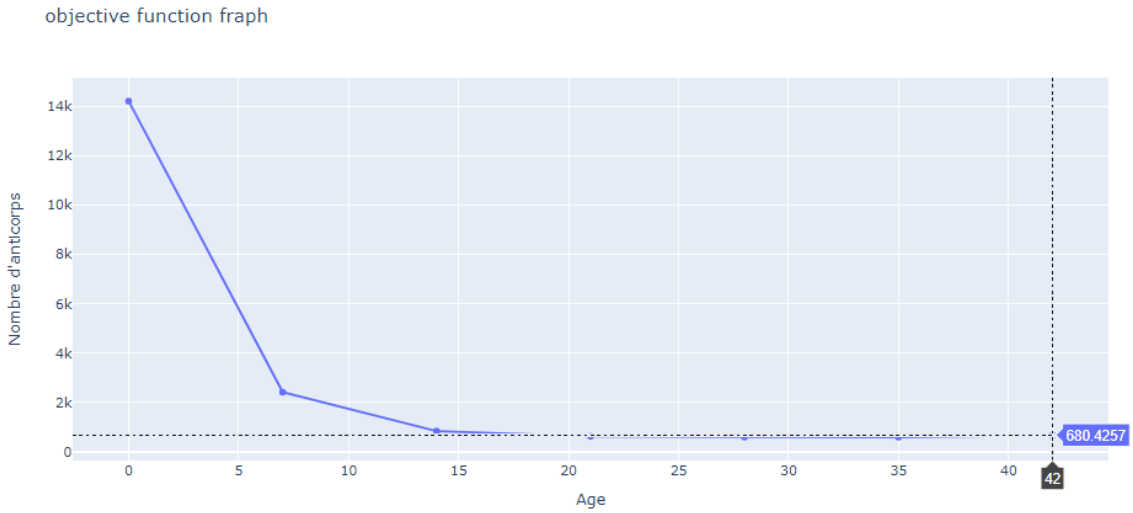

Figure 17: Variation of mean titers of maternal anti-H9N2 antibodies in response to vaccination at 21 days

As the plot shows if we vaccinate at 21 day after 14 days of latency we will star to have an immune response and if we take the example of 42 days we will have an antibody level of 680,42 units.

Comparing the two results for the FLUNPS kit, we can see that the first model recommended earlier vaccination (at a younger age) than the second model.

4. Limitations and recommendations

In summary, our study has provided valuable insights into the optimal age for vaccination against H9N2. However, there are limitations to consider, such as variations between test kits and individual immune response factors. To overcome these limitations, future research should focus on evaluating different test kits, considering individual immune response, and exploring alternative modeling approaches. Additionally, expanding the dataset and collecting more comprehensive data will improve the accuracy of our findings. By addressing these limitations, we can refine our understanding of optimal vaccination strategies against H9N2 and enhance disease control measures.

Conclusion

In summary, this research delved into the critical question of determining the optimal age for H9N2 vaccination in poultry. Leveraging two distinct mathematical models, we have arrived at valuable insights that have significant implications for poultry health management, disease control, and public health.

Our first model, founded on the principles of antibody degradation and a predefined cut-off point, revealed that the majority of chicks achieve the required antibody activity level within approximately 19 days. For the FLUH9S kit, the optimal age stood at 20 days, while the FLUNPS kit suggested an optimal age of 18 days.

The second model, involving an optimization problem that accounts for both antibody degradation and the immune response, pointed to an optimal vaccination age of 21 days. Moreover, this model estimated the antibody level to be 596 units for the FLUNPS kit.

These findings underscore the profound impact of vaccination timing on the effectiveness of H9N2 virus protection. By diligently examining maternal anti-H9N2 antibody titers and employing sophisticated mathematical modeling approaches, our study lays the foundation for the development of precise vaccination strategies.

As we move forward, our research extends its scope. We are actively developing a continuous-time Markov chain model to delve deeper into the time-dependent transitions between different states. This endeavor promises a more comprehensive understanding of optimal vaccination age. Simultaneously, we aim to enhance accessibility through a user-friendly graphical interface, designed to facilitate data input, result visualization, and decision-making for healthcare professionals and policymakers alike.

Looking ahead, future research must encompass the expansion of our database and the acquisition of additional data, including demographic information. These steps are pivotal in gaining a more holistic grasp of optimal vaccination age and enabling meaningful comparisons between models. The exploration of advanced modeling techniques remains a priority, as it holds the key to heightened prediction accuracy and robust vaccination schedule recommendations.

Furthermore, the evaluation of the long-term efficacy of the immune response at different ages emerges as a fundamental task. This assessment will offer vital insights into the sustained impact of vaccination strategies and their overall effectiveness.

In conclusion, while our study has shed valuable light on the optimal age for H9N2 vaccination, we acknowledge its limitations stemming from data constraints. Therefore, we underscore the need for ongoing research and data collection to validate and refine our findings. By addressing these limitations and pursuing the outlined future research

directions, we can propel vaccination strategies forward, enhance disease control measures, and safeguard public health.

This revised conclusion maintains a technical tone and structure while encapsulating the key points and implications of your research.