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Available vaccines, antigenic distance, and analysis of Bulgarian HPAI viruses in the light of possible vaccination

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Summary

Avian influenza is still of a big concern for poultry production. The last year many European countries considered some strategies for vaccination. France was the first country in Europa who started vaccination program since the autumn of 2023. The aim of this study is to present the importance of knowing the genetic characteristics of current strains circulating in Bulgaria. Knowing their origin gives us the knowledge to predict and choose the future management of the outbreaks. The landscape of avian influenza vaccines is a dynamic process, with ongoing research and development to address emerging strains and improve vaccine technologies. That is why we should be prepared with methods and techniques to control the disease. Additionally, specific details can vary, and recommendations may change based on the evolving nature of avian influenza viruses.

Key words: vaccines, influenza, poultry

Introduction

Vaccines. Avian influenza refers to the disease caused by Influenza A virus. Naturally, these viruses are spread among wild and domestic birds and some mammals, and cause sporadic infections in humans. Avian influenza viruses are divided into two types based on their pathogenicity, high pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI). In recent years, Europa has faced an increase in HPAI outbreaks in poultry and cases in migratory waterfowl birds. Areas with high concentrations of farms have faced serious problems and economic losses during epidemic seasons. Circulating viruses are the consequence of multiple reassortant events. Many countries are looking for solutions to prevent and control the disease, and to decrease the damage in the poultry industry. Vaccination is a popular tool for preventing many diseases in animals and humans. Nevertheless, finding a fully effective vaccine that can protect poultry from HPAI completely is a challenging task. An ideal avian influenza vaccine should be: effective in stopping transmission, usable in multiple avian species, for mass application, applied at one day of age, provide long-lasting protection after a single dose, antigenically close to circulating strains, induce a long protective immune response, allows easy identification between infected and vaccinated birds by serological diagnostic means

Various vaccines, including traditional and next-generation vaccines, have been developed to protect against influenza viruses. The traditional vaccines could be based on inactivated viruses or vectors and next-generation vaccines could be DNA or RNA.

The inactivated AIV vaccines are the most widely used up to date. That is why the only authorized vaccine against HPAI in the EU is Nobilis AI H5N2. It contains an inactivated avian influenza virus strain H5N2 A/duck/Potsdam/ 1402/86. The vaccine is approved only for

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chickens (experimentally in Pekin ducks, and turkeys), application is injected intramuscularly or subcutaneously (from 14 days onwards), has a wide antigenic distance, and was not efficacious to stop transmission in experimental settings.

The inactivated vaccines are produced by the most popular technology by growing the virus in chicken embryonated eggs or cells. After harvesting the virus is purified and emulsified with oil adjuvant. Formaldehyde or β-propiolactone is used for the inactivation of the vaccines. Formaldehyde works by manipulation of the protein. However, there are some disadvantages of formaldehyde, its toxicity, and the changes in antigen epitope. β-propiolactone passes through the viral membrane and denaturates nucleic acids, which ensures that the virus is no longer capable of replicating or causing diseases. Inactivated vaccines with β-propiolactone have been widely used and have a good safety profile.

Vector vaccines used live or inactivated vectors such as Fowlpox virus, Newcastle disease virus, adenovirus, duck enteritis virus, herpesvirus of turkey, baculovirus, infectious laryngotracheitis, Salmonella, lactobacilli. This type of vaccination is useful for the poultry industry because it includes strains against several diseases.

Recombinant technology and nucleic acid technology nowadays become very popular, because they allow smooth adaptation to the circulating strains. That is a big advantage compared with whole virus vaccines. Recombinant vaccines for avian influenza used insertion and transference of the DNA section responsible for encoding the antigen. These vaccines used harmless vectors to carry genetic material and stimulate an immune response. Virus-like particles are a type of recombinant vaccine. These particles can be produced from different expression systems such as yeast, bacterial, insect, or animal cells. They mimic the structure of the avian influenza virus, triggering the immune response without using infectious viruses.

There are only two vaccines for avian influenza based on nucleic synthesis (EFSA Journal, 2023). One is RNA replicon, with compromised replication of the acid, and another one is DNA vaccine. The RNA replicon vaccine is engineered to carry genetic information encoding antigens specific to the avian influenza virus. During the replication in the cells, RNA replicon produces the antigens of the virus. The presentation of viral antigens on the surface of the host cells triggers an immune response and the production of antibodies. The DNA vaccine involves using genetic material, specifically DNA. They carry genetic material that codes for specific antigens of the avian influenza virus. These antigens are the proteins that the immune system recognizes as foreign and cells transfected with the DNA start to express the viral antigens. This mimics a natural infection, triggering an immune response. These vaccines based on nucleic synthesis are relatively easy to design and produce and induce a strong immune.

Avian influenza in Bulgaria. All viruses isolated so far in Bulgaria originate from A/Goose/Guangdong/1/96. It is a strain of the influenza A subtype H5N1 virus that was first detected in a goose in Guangdong in 1996, creating a Z genotype (also called the "Asian lineage" HPAI A H5N1) that has spread globally and until now.

For the first time in Bulgaria, highly pathogenic influenza (HPAI) H5N1 was detected in 2006 (Goujgoulova et al. 2007). The phylogenetic analysis of the viruses from the epidemic during this period shows a close relationship between the Bulgarian isolates and those from Europe, the Middle East, and Africa (Goujgoulova, G., 2010). The viruses belong to clade 2.2. In the spring of 2010, there was a new penetration of HPAI H5N1 in the region of Varna, and the virus was clade 2.3.2.1c (Marinova-Petkova et al. 2012). The virus was found only in one wild bird (Buteo Buteo) and did not appear again until 2015. In 2011 several low-pathogenic

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H5 viruses were detected in wild and domestic ducks, designated as EA_nonGsGD (MarinovaPetkova, A., 2012). In the winter of 2015, HPAI H5N1 2.3.2.1c had a new introduction in the country, affecting first pelicans in the Poda, region of Burgas, and after a few days in a farm with chickens located in the same region. Later it was found in other wild and synanthropic birds again in this part of Bulgaria, as well as in pelicans in the Srebarna reserve, region Silistra.

In the next few years, no other infiltrations of HPAI were detected in Bulgaria. In December 2016 the first outbreak of HPAI H5N8 was observed. After that, the virus spread rapidly in different administrative areas. Phylogenetic analysis shows that the viruses from Bulgaria originated from European H5N8 strains of lineage 2.3.4.4b. The Bulgarian viruses isolated in 2017–18 form two distinct clusters originating from different progenitors of the 2.3.4.4b virus. The clusters are structured according to the geographical characteristics of their origin. The strains from Dobrich form one cluster in the northeast, those from the central Bulgarian regions (Plovdiv, Haskovo and Stara Zagora) form a second one, and the viruses from the eastern and northwestern regions (Yambol, Sliven and Vidin) are also included. The study reports the identification of two separate virus introductions, one in the northeastern region of Dobrich and another in central and eastern Bulgaria. The duck sector has been suggested to have played a critical role in the spread and maintenance of the HPAI H5N8 virus in the country (Venkatesh et al. 2020). Genetic analysis shows that the transmission of the virus to poultry is most likely due to domestic ducks. H5N8 viruses are likely to have been endemically maintained in the poultry sector. However, matrix protein sequencing demonstrated relatedness to isolates from multiple species (ducks, swans, pheasants) in different countries (Netherlands, England, Hungary).

During the period March 2019-February 2021 HPAI H5N8 2.3.4.4b is re-introduced in the country, with one new H5N2 subtype likely emerging from a local reassortment event. A source of cryptic spread and maintenance of HPAI H5N8 comes from the domestic duck sector, with fattening farms playing a major role. The analysis shows the movement of viruses in Bulgaria between farms, but the viruses are largely regionally determined. There is no data on movement between regions, for example between Dobrich in the northeast and Plovdiv in Central Bulgaria. However, the connections between Plovdiv and Eastern Bulgaria (Yambol, Sliven), which are geographically relatively close, are clear. Indirect transmission is also observed, the virus from the Vidin region appears to be closely related to viruses found in Central Bulgaria, indicating a possible link between these regions through wild birds or overland translocation (Venkatesh et al. 2020).

In November 2021 H5N1 appeared again and until now it remains the dominant strain distributed on the territory of the country. Phylogenetic analysis shows that, like H5N8, it belongs to clade 2.3.4.4b.

Materials and methods

Sequences of viruses. The used sequences have two origins. The old ones, from 2007 are part of the Ph.D. thesis of Dr. G. Goujgoulova (Goujgoulova, G., 2010). The other sequences of Bulgarian isolates and the vaccines are found at Global database for influenza gene sequences (https://gisaid.org/). For the current analysis, it was used only HA sequences.

Vaccines. We used sequences of six viruses, which are used in seven commercial vaccines against avian influenza. They are shown in Table 1.

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Table 1.Type of vaccines and strains, which they contain. https://www.efsa.europa.eu/en/news/vaccination-poultry-against-highly-pathogenic-avianinfluenza-available-vaccines-and

Name of the vaccine	Strain	Type of the vaccine
Duck H5-SRVvaccine	H5N8 A/duck/France/161108 h/2016	Replicon
Vaxigen Flu	H5N8 A/green-winged teal/Egypt/877/2016	Inactivated full virus
Volvac B.E.S.T. AI +ND	H5N1 A/duck/China/E319-2/2003	Subunit
Vectormune AI	H5N1 A/mute swan/Hungary/4999/2006	Live vector
Nobilis Influenza	H5N2 A/duck/Potsdam/1402/86	Inactivated full virus
CEVAC Flu-Kem H5N2	H5N2 A/Chicken/Mexico/232/94/CPA	Inactivated full virus
Volvac AI KV H5N2	H5N2 A/Chicken/Mexico/232/94/CPA	Inactivated full virus

Evolutionary analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (1). Evolutionary analyses were conducted in MEGA11 (2). The tree was obtained automatically by applying Neighbor-Join and BioNJ algorithms using the Tamura-Nei model and then selecting the topology with a superior log likelihood value.

Estimates of Evolutionary Divergence between Sequences. Analyses were conducted using the Maximum Composite Likelihood model (1) using MEGA11 (2). This analysis involved 20 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding.

Results

A phylogenetic analysis of the hemagglutinin of the viruses in Bulgaria from 2021 was made. They were compared with some vaccines available on the market (Table 1.). The selection of vaccines used in the study was based on preliminary trials in France and the Netherlands. It should be noted that the obtained results may change after including the sequences of the 2023 isolates.

What the genetic analysis shows for the CEVAC Flu-Kem H5N2 and Volvac AI KV H5N2 vaccines, which are based on the H5N2 A/Chicken/Mexico/232/94/CPA strain, is that it is antigenically distant from all Bulgarian isolates. A/duck/Podsdam/1986/H5N2, which was used in Nobilis Influenza H5N2, clustered with LPAI H5N8/N2 from 2011. Closest antigenically to the 2021 HPAI H5N1 viruses are Duck H5-SRVvaccine and Vaxigen Flu H5N8.

The evolutionary divergence between the sequences of fourteen 2021 H5N1 strains and selected sample vaccines was presented in Figure 2. Vaccines used strain H5N2 A/Chicken/Mexico/232/94/CPA have the greatest number of evolutionary divergences. After them is H5N2 A/duck/Potsdam/1402/86, A/swan/Hungary/4999/2006 and H5N1 A/duck/China/E319-2/2003 and. The strains with minimal antigenic distance are A/duck/France/161108 h/2016 and A/green-winged teal/Egypt/877/2016.

A

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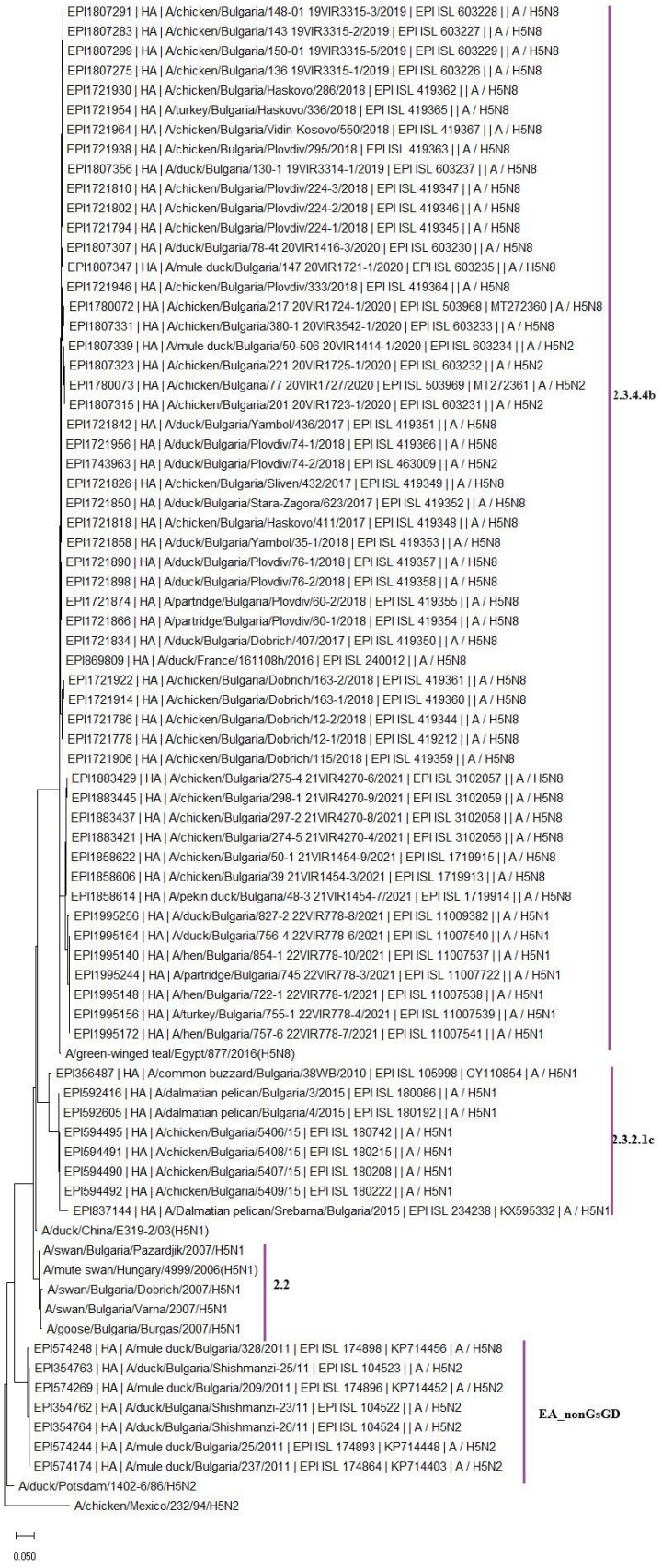

B

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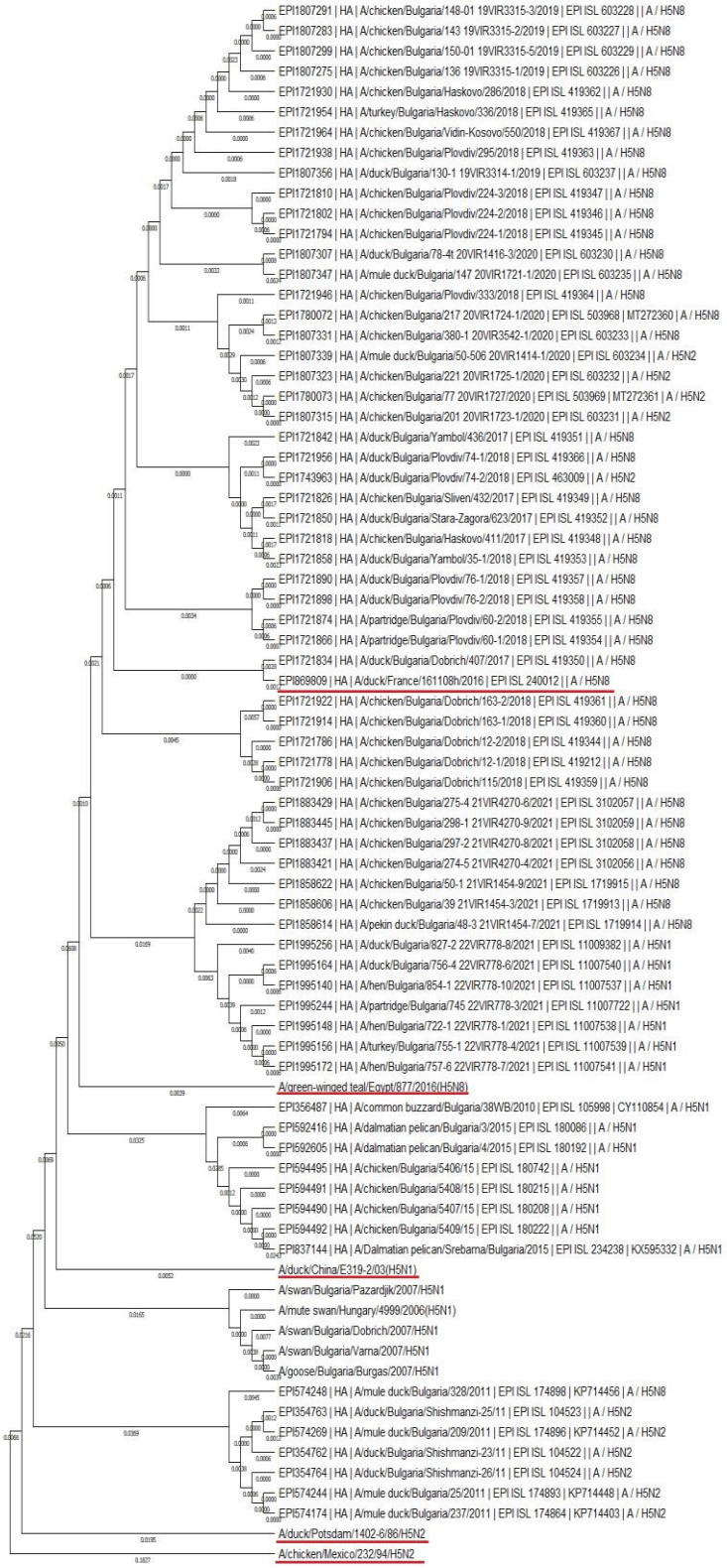

Figure 1. Maximum Likelihood phylogenetic tree of the HA gene. A. Clusters of Bulgarian isolates and used vaccines. B. Phylogenetic tree with branch length.

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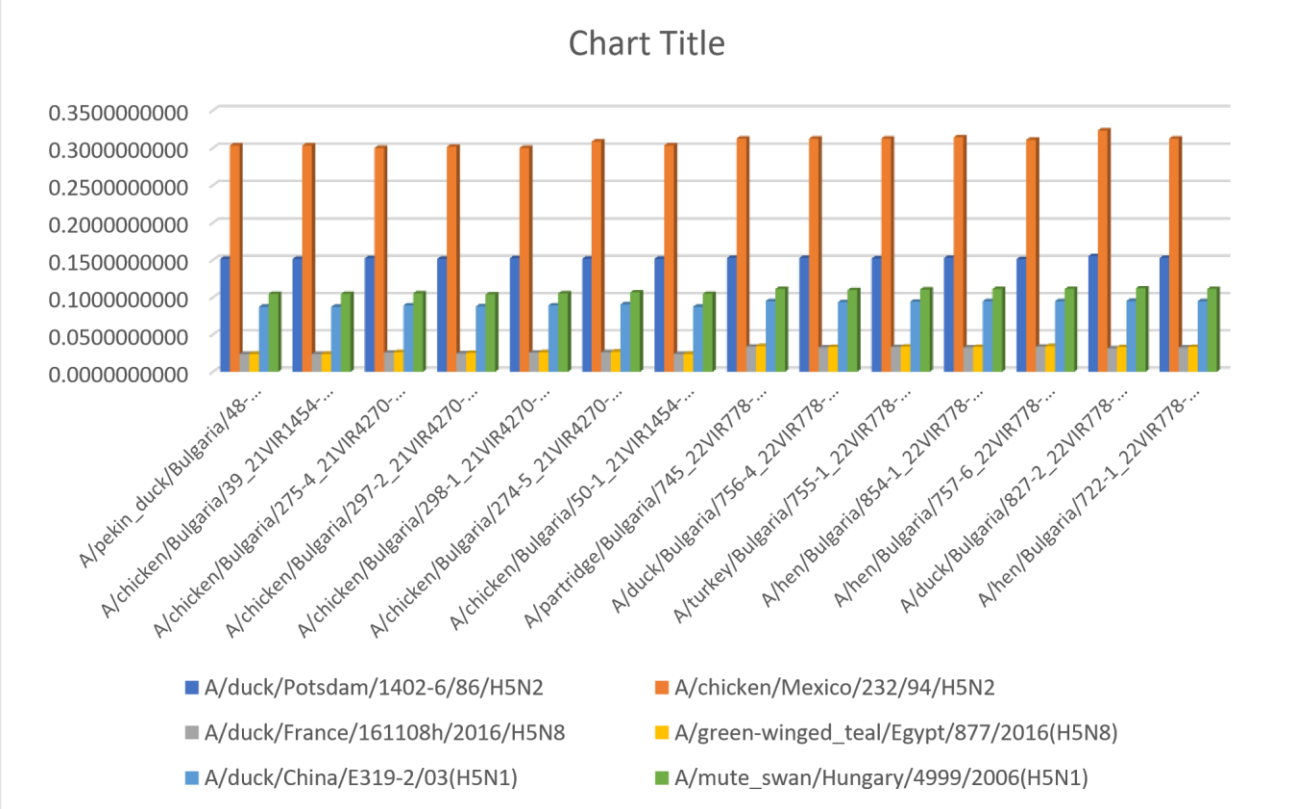

Figure 2 . Estimates of Evolutionary Divergence between Sequences. The number of base substitutions per site from between sequences is shown.

Discussion

Previous experience with vaccinations carried out outside the EU has highlighted the importance of minimizing the antigenic difference between the strains used in the vaccine and those in the field. It is possible that the low protection due to the high antigenic difference of the vaccine strains from the circulating viruses can be overcome by strengthening the immune responses using effective adjuvants and high potency vaccines (eg vaccines with a high content of immunogenically relevant antigens) or a combination of vaccines, primarily stimulating humoral immunity (eg inactivated or subunit vaccines) with vaccines inducing cellular immunity against conserved epitopes (eg live vector vaccines) (EFSA, 2023). Some live vectored vaccines, such as HVT-vectored vaccines can be given earlier, because they are less affected by maternal immunity, which is the main problem with NDV-vectored vaccines. On the other hand, if it used vaccines with the same vector, that will raises the possibility of reduced effectiveness due to vaccine interactions. For example, when it is selected HVT vector vaccines used in parent flocks or laying hens eg Marek, NDV, IBD. So when preparing a vaccination program, it should be done so that it contains only one vector vaccine (Germeraad et al. 2023). Another consideration is off-label use of vaccines in species different than chicken. The effectiveness could drop because of inefficient replication, for example, of HVT in ducks.

Our analysis shows that the greater the number of evolutionary divergences, the further antigenically the vaccine is from the field strains. This only confirms what was said in the EFSA opinion (EFSA, 2023) that the Duck H5-SRV vaccine is antigenically closest to Bulgarian isolates from 2021 due to the presence of H5 from clade 2.3.4.4b in the vaccine composition.

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France chose the Volvac B.E.S.T vaccine for its vaccination program. It is a recombinant Baculovirus-based subunit vaccine. France had previously conducted trials of this as well as other vaccines, and it is likely that the results of these trials led to their selection. Another important point is the availability of the DIVA test. IDvet offers ELISA DIVA test ID Screen Influenza A Nucleoprotein Indirect ELISA. for recombinant vaccines: HVT –H5 (Vector with Herpesvirus H5N1); VLP-H5, virus-like particles prepared in mammalian, insect, plant expression systems, RSV-H5.

From a previous analysis of the distribution of HPAI, it can be concluded that 20162018 H5N8 in Bulgaria has been maintained probably endemic in the poultry sector. A source of cryptic spread and maintenance of the virus comes from the domestic duck sector, with fattening farms playing a major role. At that time, France and Hungary were also affected (Guinat et al., 2019). These countries, together with Bulgaria, are the main producers of foie gras in Europe and maintain trade links connecting duck farms (transportation of ducklings and eggs) in all three countries (Delpont et al., 2021). The epidemics in 2021-2022 and 2022-2023 show global spread affecting all continents (Lambert et al., 2022). This means that controlling the disease will require even more stringent measures. The phylogenetic analysis that we have done is just an example and only wants to show the need to do it before choosing a vaccine.

The choice of vaccine should be consistent with the antigenic proximity between the field strain and the virus strain. In the case of vaccination selection, strict monitoring of mutations in the virus genome is necessary, which requires timely sequencing of each field strain. The choice of vaccine should be consistent with the presence of a DIVA strategy (Marangon et al, 2006). With some vaccines, it is possible to use Neuraminidase Inhibition Reaction or ELISA. It follows from this that once a vaccine is selected, it is necessary to ensure the control of the vaccination with the appropriate laboratory test.

Conclusions

EFSA recommends vaccination in the most susceptible poultry species in regions with a high risk of transmission to reduce the number of infected and destroyed farms and the duration of the outbreak. The most sensitive birds to the Influenza A virus are chickens. In Bulgaria, hen farms are concentrated throughout the country. Comparing the HPAI outbreak over the years, the wild bird migration, and the livestock density, three areas of concentration of all risk factors are identified. These are reg. Plovdiv, Stara Zagora and Haskovo.

If the aim is to minimize the number of outbreaks and the duration of the epidemic, EFSA recommends preventive vaccination of the most susceptible species in areas with a high risk of transmission. For areas with high risk of virus introduction from wild birds and low density of holdings, preventive vaccination is also recommended to reduce the number of outbreaks. Based on the above, three variants of the vaccination strategy can be proposed:

-Precautionary vaccination only in the region Plovdiv, Haskovo and Stara Zagora in all animal breeding sites with ducks and chickens (ducks - 1,509,832 pieces; chickens - 601,606 pieces)

- Protective vaccination of laying hens only, covering the whole country (the total number of birds is 4,973,793).

- Preventive vaccination only in the Zoo in the city of Sofia.

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