Hafez M. Hafez
Awad A. Shehata *Editors*

Turkey Diseases and Disorders Volume 2

Infectious and Nutritional Diseases, Diagnostics and Control Strategies

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Preface

The global turkey business strives for increased production, higher quality, and competitive pricing. Turkey production has increased in recent decades due to the progress made in artificial brooding, genetics, nutrition, and management. However, the rising demand for turkey meat requires a consistent, practical, and goal-oriented healthcare system to minimize and control the emergence and spread of infections in turkey farms. Nevertheless, the production and health of turkeys are also being impacted by various factors and problems. Among these factors are intense global competition between producing countries and permanent changes in social, political, and consumer perceptions regarding food safety, animal welfare, and environmental protection. Several human foodborne infections are linked to poultry and poultry products, causing a serious challenge because it is difficult to control. Moreover, contamination of turkey meat and products with antibiotic-resistant bacteria is a constant public health hazard. The loss of consumer confidence and trust in turkey meat product safety and quality will also be a major concern.

The current and future turkey health concepts should cover the control of diseases in birds and the relationship between birds' health, welfare, and environmental protection. Additionally, the emergence and re-emergence of infectious turkey diseases will remain an important and never-ending challenge. Only a few authorized pharmaceuticals and veterinary products are available to treat turkeys. Developing efficient vaccines and natural antimicrobials against bacterial infections will reduce antibiotic use and resistant bacteria's development. Genetic selective breeding to improve production traits and health is a long-standing goal of the turkey industry. Furthermore, rearing technology, management, and feeding will help maintain the birds healthy and comfortable. Finally, all other partners involved in the production chain, including farmers, veterinarians, and stockholders, need to collaborate to meet consumer expectations for high-quality and safe products.

The book *Turkey Diseases and Disorders* aims to address the main challenges facing turkey production and is organized into two volumes: Volume 1 covers the main bacterial and fungal diseases of turkeys in 22 chapters. Volume 2 covers viral and parasitic diseases and nutritional disorders in 20 chapters. The book is designed to be a handbook for undergraduate students and a valuable source for researchers, practical poultry specialists, and nutritionists. Additionally, this book may be instrumental as a guide for production and health problems in turkeys. At the end of each chapter, we provide the reader with selected literature that covers the topic.

Comprehensive citation of the references is minimized, and the presentation of literature data is based on the interpretation or correlation of research findings.

This book can serve as a textbook, a research reference, and a valuable guide to the knowledge of turkey management and diseases. We hope that readers will find this book useful and interesting to read.

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Editors and Contributors

About the Editors

Awad A. Shehata gained his bachelor's degree in veterinary medicine from the Faculty of Veterinary Medicine, Alexandria University, Egypt, and his license as veterinarian from Ludwig-Maximilians-University, Munich, Germany. In 2005, he obtained a master's degree in avian diseases at the Faculty of Veterinary Medicine, Sadat City University, Egypt. In 2011, he completed his Ph.D. (Dr. med. vet.) in virology at the Faculty of Medicine, Leipzig University, Germany. In 2015, he obtained his Dr. habilitatus (Dr. med. vet. habil.) in bacteriology and poultry diseases at Leipzig University, Germany. In 2022, he was honored with the title of professor of avian diseases from Sadat City University, Egypt. Besides his academic work at several universities, Dr. Shehata has six years of industrial experience in vaccine production and the development of alternatives to antimicrobials. He is a certified quality manager, auditor, project manager, European Business Competence License level (EBCL), Good Manufacturing Practice (GMP), FELASA-B and -C, and phytotherapist. Dr. Shehata's research interests lie mainly in developing alternatives or complementary to antimicrobials and diagnostic strategies and studying avian pathogens' epidemiology and molecular epidemiology. Alternatives include developing and evaluating recombinant vaccines, live attenuated bacterial vaccines, inactivated vaccines, probiotics, and prebiotics. Dr. Shehata teaches avian diseases and microbiology courses in English and German at several universities worldwide. He is currently a project leader at the Structural Membrane Biochemistry, Technical

University of Munich, Garching, Germany. His research focuses on the analysis and mechanism of action of natural products, metabolic pathways, and fluxes in various organisms using 13C-labeling experiments and profiling.

Hafez M. Hafez was Head of the Institute of Poultry Diseases of the Free University in Berlin from 1997 until 2016. He is currently a guest senior professor at the same institute. Hafez gained his Master of Veterinary Science (MVSc) at the Department of Poultry Diseases from Cairo University, Egypt, in 1975. In 1981, he completed his Doctorate Degree (Dr. med. vet.) at the Department of Poultry Diseases, Giessen University, Germany. In 1994, he finished the Habilitation (Dr. med. vet. habil.) thesis at the Department of Poultry Diseases, Munich University, Germany. Dr. Hafez has been recognized as a veterinary poultry specialist since 1982, veterinary microbiology specialist since 1989, and veterinary animal hygiene specialist since 1996. He became the diplomat of the European College of Veterinary Public Health (Dipl. ECVPH) in 2005 and the European College of Poultry Veterinary Science (Dipl. ECPVS) in 2009. He is currently the honorary life president of the World Veterinary Poultry Association (WVPA). He was the past-president of the ECPVS, the chairman of the poultry scientific committee of the German Veterinary Chamber, the chairman of the German branch of the WVPA, the chairman of Working Group 10 (Turkey), and European Branch of World Poultry Science Association (WPSA). Besides, he has been an honorary professor at the University of Hohenheim, Germany, since 1996 and an honorary professor at Alexandria University, Egypt, since 2009. Since 2015, he has been an advisor to the Arab Federation for Food Industries (AFFI). Dr. Hafez' research interest focused on poultry disease diagnosis and control in general and food-borne diseases, management, animal welfare, and hygiene.

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Part I

Viral Diseases

Avian Influenza

1

Awad A. Shehata and Hafez M. Hafez

Abstract

The first report on the isolation of the influenza virus from turkeys with respiratory signs was in the 1960s in the United States. Since then, several outbreaks caused by low-pathogenic (LP) and highly pathogenic avian influenza (HPAI) subtypes, that is, H1, H3, H5, H6, H7, and H9, have been observed in many countries worldwide. Differences in susceptibilities between turkeys and chickens have been identified. It was found that H7N2 was more infectious for turkeys than chickens. Sinusitis is a common sign in turkeys infected with LPAI. In addition, turkeys play an important role in the evolution of avian influenza viruses for several reasons: (i) Turkeys have both avian- and human-type receptors, making them highly probable mixing vessels for avian influenza viruses. Both avian-type and human-type receptors are expressed in the nasal cavity, lung, kidney, esophagus, and intestine. (ii) Turkey breeders especially can also be infected with swine influenza viruses such as H1N1, H1N2, and H3N2, causing a severe drop in egg production and severe economic losses as well as increasing the probability of reassortments and virus evolution in turkey hosts. (iii) Interspecies transmission of swine influenza viruses to turkeys is common. Interspecies transmission between ducks to turkeys has been reported but less frequently. Mixing different poultry species and outdoor rearing could favor the adaptation of LPAI viruses and pose a serious health risk.

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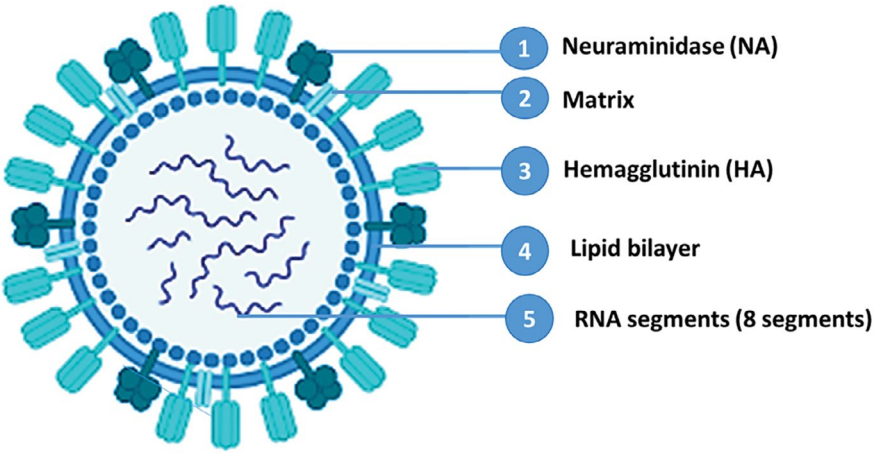

Fig. 1.1 Avian influenza virus (Generated by Biorender)

Keywords

Turkeys · Influenza · HPAI · LPAI · Virus evolution · Vaccination

1.1 Etiology

AI viruses are members of the *Orthomyxoviridae* family. Four types of influenza viruses, A, B, C, and D, are known within this family, based on nucleoproteins and matrix proteins (Kuhn et al. 2020). The structure of avian influenza is illustrated in Fig. 1.1. The virus genome is a single-stranded segmented RNA; the genome of influenza A and B viruses comprises eight gene segments, while the influenza C and D genome comprises seven segments (Abdelwahab and Mettenleiter 2023).

The eight segments of influenza A viruses encode at least ten viral proteins: PB2, PB1, PA, HA, NP, NA, M1, M2, and NEP are included in the virion, while the non-structural protein NS1 is expressed only in host cells after infection. Type A viruses can infect birds, humans, and mammals such as horses, pigs, seals, and whales. The virus is RNA, enveloped, and sensitive to ether, chloroform, and different chemical disinfectants. Type B and C affect only humans. Type D infects a broad range of mammalian species.

1.2 Influenza Pandemics

AIV causes periodic epidemics in humans, horses, pigs, seals, whales, and several birds (Swayne et al. 2020).

(i) The Russian flu pandemic emerged between 1889 and 1890 in Russia. It was likely caused by H3N8 and H2N2 strains (Ryan 2008).

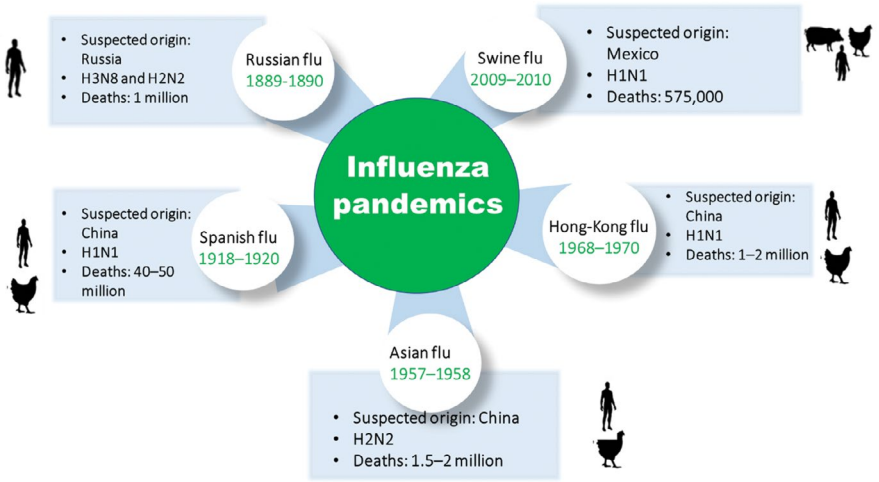

Fig. 1.2 Pandemic and epidemic events of influenza (Generated from Parvin et al. 2022)

(ii) Spanish flu was the first documented influenza pandemic in humans. About 500 million people were infected, and about 50 million deaths were reported over 2 years. The cause of Spanish flu is H1N1 of avian origin, which emerged in four waves during 1918–1920 (Bassareo et al. 2020).
(iii) Asian flu and Hong Kong flu pandemics emerged in 1968 and 1970 and were caused by H2N2 and H1N1 viruses. The two viruses had reassortment events comprising human and avian-origin gene segments (Martini et al. 2019).
(iv) Swine flu was caused by a novel H1N1pdm09 virus in 2009 (Riley et al. 2011; Dawood et al. 2012). This virus emerged from triple reassortment between avian, swine, and human influenza viruses (Tewawong et al. 2015). Various pandemic and epidemic events of influenza are illustrated in Fig. 1.2.

1.3 Evolution of Influenza Viruses

There are three mechanisms of influenza viruses' evolution:

(i) **Antigenic drift:** The virus genome experiences a single mutation that alters the amino acid sequence. The reason for these mutations is a lack of the ability to proofread RNA-dependent RNA polymerase (RdRP), which causes a rate of 10^{-3} and 10^{-4} integration of false nucleotides (Drake 1993; Shao et al. 2017). By altering or hiding the immunogenic epitopes of a circulating virus, it may cause immunological escape. The primary cause of vaccination failure in humans and poultry is antigenic drift, which necessitates regular updates to influenza virus vaccines (Grund et al. 2011; Wen et al. 2021).
(ii) **Antigenic shift (Reassortment):** When a host cell is co-infected with numerous viruses, the genome segments may be shifted to generate progeny viruses with novel genome combinations (Marshall et al. 2013). Antigenic shift

between avian, swine, and human influenza viruses has been reported, leading to the emergence of new subtypes. Although pigs serve as a vessel for the mixing of influenza viruses, other host species, such as turkeys and quails, as well as humans, may also participate in this way; therefore, pigs are not the only species that participate in the production of reassortant influenza viruses (Hennig et al. 2022). More recently, potential "mixing vessels" based on the distribution of avian and human sialic acid receptors have been categorized into: (i) high probable mixing vessels hosts, such as humans, pigs, minks, ferrets, seals, dogs, cats, birds, turkeys, chickens, quails, and ducks; (ii) medium probable mixing vessel hosts such as nonhuman primates, raccoons, camels, pikas, horses, and zoo animals, including tigers and lions; and (iii) low probable mixing vessels hosts such as foxes, bats, and whales (Abdelwahab and Mettenleiter 2023).

(iii) **Recombination:** Parts of the influenza gene segments, or host cellular RNA, are integrated into other gene segments, known as homologous recombination and/or nonhomologous recombination, respectively. HPAI emerged from LPAI due to recombination in the HA cleavage site (Gultyaev et al. 2021).

1.4 Subtypes and Pathotypes of Avian Influenza

To date, there are 18 H subtypes and 11 N subtypes of Influenza A viruses. Avian influenza viruses (AIVs) contain H1 to H16 and N1 to N9 subtypes, while H17N10 and H18N11 are detected or isolated only in bats (Gamblin and Skehel 2010; Suarez 2016). According to the pathogenicity, influenza viruses are classified into LAPI and HPAI (Table 1.1).

Representatives of all the different subtypes of Influenza A viruses have been isolated from several species of birds, mainly from aquatic species such as ducks, geese, and gulls. The viruses are known to have a high mutation rate. The mutation

Table 1.1 Features of high pathogenic (HPAI) and low pathogenic (LPAI)

Criteria	HPAI	LPAI
The cleavage site of HA0	Multiple basic amino acids, such as lysine and arginine	Monobasic amino acid
HA0 cleavage	Ubiquitous cell proteins are found in most cells of the body	Only trypsin-like proteases found in the respiratory and digestive systems
Virus replication	Pantropic (all cells and all organs)	Epithelial cells of respiratory and digestive tracts
Virus replication in cell culture	Without trypsin	With trypsin
Subtypes	H5 and H7[a]	Other subtypes
Lethality in 4–6 weeks chicks (IV-infection)	6–8/8	

[a] H5 and H7 are usually HPAI with few exceptions

of the virus and recombinations between strains lead to ongoing dynamic changes in the virus surface structures (Swayne et al. 2020). Several features are used to describe new influenza viruses, including antigenic type (A, B, C, D), animal host, geographical location (city, state, or country), laboratory or reference ID, the year of isolation, and HA and NA types. An example is A/chicken/Egypt/SCU20/2014 H9N2.

1.5 Avian Influenza in Wild and Domestic Birds

AIV subtypes H1 to H11 and H13 have been detected and/or isolated from domestic birds. The most frequently isolated subtypes in domestic birds are subtypes H5Nx, H6N2, H7N3, H7N9, and H9N2. However, subtypes H12 and H14-H16 have not yet been detected in domesticated birds. Mixed infections with several influenza subtypes were reported. Mixed viral infections were observed in both broiler and layer chickens. The detected triple H5N1, H9N2, and H5N8 influenza co-infection raises the concern of potential AI epidemic strain emergence (Shehata et al. 2019). AIVs that are highly pathogenic in ducks are also highly pathogenic in chickens, but the reverse is not true. Most HPAIV H5/H7 strains in ducks are non-virulent, unlike in chickens and turkeys. Mallard ducks are known to be the primary carriers of avian influenza viruses, although several H5N1 and H5N8 viruses are also highly virulent in mallards. Muscovy ducks are more sensitive than Pekin ducks (reviewed in Abdelwahab and Mettenleiter 2023). The diversity of host-specific influenza viruses is shown in Tables 1.2 and 1.3.

Table 1.2 Diversity of host-specific influenza viruses. The H17N10 and H18N11 subtypes were identified in bats

Subtype	Human	Horse	Pig	Wild birds	Domestic birds	Spill over to other animals*
Diversity of HA						
H1	■		■	■	■	
H2	■			■	■	
H3	■	■	■	■	■	
H4				■	■	
H5	■		■	■	■	■
H6				■	■	
H7	■	■	■	■	■	
H8				■	■	
H9	■			■	■	
H10	■			■	■	
H11				■	■	
H12				■		
H13				■	■	
H14				■		
H15				■		
H16				■		
Diversity of NA						
N1	■		■	■	■	■
N2	■			■	■	
N3			■	■	■	
N4				■	■	
N5				■	■	
N6			■	■	■	
N7	■	■		■	■	
N8		■		■	■	
N9				■	■	

* Mice, ferrets, monkeys, tigers, cats, dogs, marten, donkeys, and civet cat.

Table 1.3 Pathogenicity of HPAI in chicken/turkeys versus ducks

	Chickens/turkey	Ducks
HPAI subtypes H5 and H7	Most strains are pathogenic and can lead to 100% morbidity and mortality in poultry	Most strains are avirulent to Mallard ducks Muscovy ducks are more sensitive compared to Pekin ducks
HPAI isolated from chickens	Pathogenic in chickens and turkeys	Not all pathogenic in ducks
HPAI isolated from ducks	Pathogenic in chickens and turkeys	Pathogenic in ducks

1.5.1 Avian Influenza in Turkeys

Turkeys are considered a bridging host for adapting wild-bird AIVs to infect poultry (Pillai et al. 2010). They are naturally susceptible to H1N1, triple reassortant H3N2 viruses. Due to the expression of both avian-type receptors in turkeys (Kimble et al. 2010), they are considered highly probable mixing vessels. Both avian- and human-type receptors are expressed in the nasal cavity, lung, kidney, esophagus, and intestine; however, only an avian-type receptor is expressed in the trachea (Pillai et al. 2010; Costa et al. 2012).

The first report on the isolation of influenza virus from turkeys with respiratory signs was in 1963 (Lang et al. 1968). In 1999, low pathogen Influenza A subtype H7N1 was isolated from turkey flocks in Italy, accompanied by a high mortality rate and a turn to virulence (HPAI) by the end of 2000 (Capua and Alexander 2004).

From December 2001 to January 2002, AI outbreaks were observed in three turkey flocks reared in the central-west region of Germany. In all cases, sudden onset of depression, decreased feed and water intake, and respiratory signs accompanied by high mortality were observed. Postmortem lesions revealed pericarditis, petechial hemorrhages in pericardial fat, fibrinous airsacculitis, lung congestion, and pneumonia (Hafez 2003).

Since 2006, HPAIV H5N1 of clade 2.2.1 infected a wide range of poultry, including turkeys, and caused severe economic losses. The virus is endemic in poultry in several countries and has diversified into two genetic clades: clade 2.2.1.1 and clade 2.2.1.2. The 2.2.1.1 clade represents immune-escape variants in vaccinated commercial poultry from 2007 to 2011.

The 2.2.1.2 clade was detected in humans, non-vaccinated backyard poultry, and, recently, commercial poultry in Egypt (Salaheldin et al. 2022). The low-pathogenic AIV A/turkey/Ontario/6213/1966 (H5N1) was isolated and proved that it is a progenitor of HPAI A/turkey/Ontario/7732/1966 (H5N9) (Ping et al. 2012).

Currently, the major turkey-producing countries have a problem at one time or another with AI (LPAI and HPAI). Although transmission from birds to humans is rare, there is a risk that these viruses may adapt and become able to infect and gain the ability to spread from person to person. Therefore, early detection and identification of human infection is of great public health importance. Focusing testing of people who develop symptoms should be tested to reduce the risk of further spread

to public health. Such investigations increase our knowledge of the zoonotic risk of influenza A viruses and provide vital evidence to help strengthen One Health responses, particularly given the unusual infection pressure in avian populations and the extensive global spread of H5N1.

1.6 Transmission and Source of Infection

The disease is transmitted directly via direct contact with infected birds and/or indirectly via contaminated equipment. Infected birds shed the virus in fecal and/or oculo-nasal discharges. Recovered flocks will intermittently shed the virus and should be considered as infected for an extended period if not all the life. The virus can survive in the contaminated environment for long periods at moderate temperatures and longer in frozen materials. The infection can be easily spread by people via contaminated shoes and clothing, crates of egg flats, vehicles, rodents, and insects that may mechanically carry the virus from infected to susceptible poultry. There is little or no evidence of vertical transmission (egg-borne infection) in poultry. However, eggshell surfaces can be contaminated with the virus. Wild and domesticated waterfowl are the primary natural reservoirs of influenza viruses. They may be infected with multiple subtypes without clinical signs, excrete the virus for long periods, and do not develop detectable antibodies.

1.7 Course of Infection

The severity of clinical signs, course of the disease, and mortality after infection with AI are incredibly variable and vary from a very mild or even inapparent form to a highly acute form, depending on the virulence of the virus, the species, age, the immune status of the host, concurrent diseases, and management.

1.8 Clinical Signs

Clinical signs after infection with HPAI may include high mortality, ruffled feathers, depression, diarrhea, sudden drop in egg production in breeder flocks, cyanosis of the snood, oedema, swelling of the head, blood-tinged discharge from nostrils, respiratory distress, incoordination, and pinpoint hemorrhages mostly seen on the feet and shanks. LPAI in turkeys is characterized mainly by respiratory manifestations.

1.9 Postmortem Lesions

The main postmortem lesions of avian influenza in turkeys are shown in Fig. 1.3. In turkeys, lesions consist of sinusitis, tracheitis pericarditis, petechial hemorrhages in pericardial fat, fibrinous airsacculitis, lung congestion, pneumonia, enlargement of

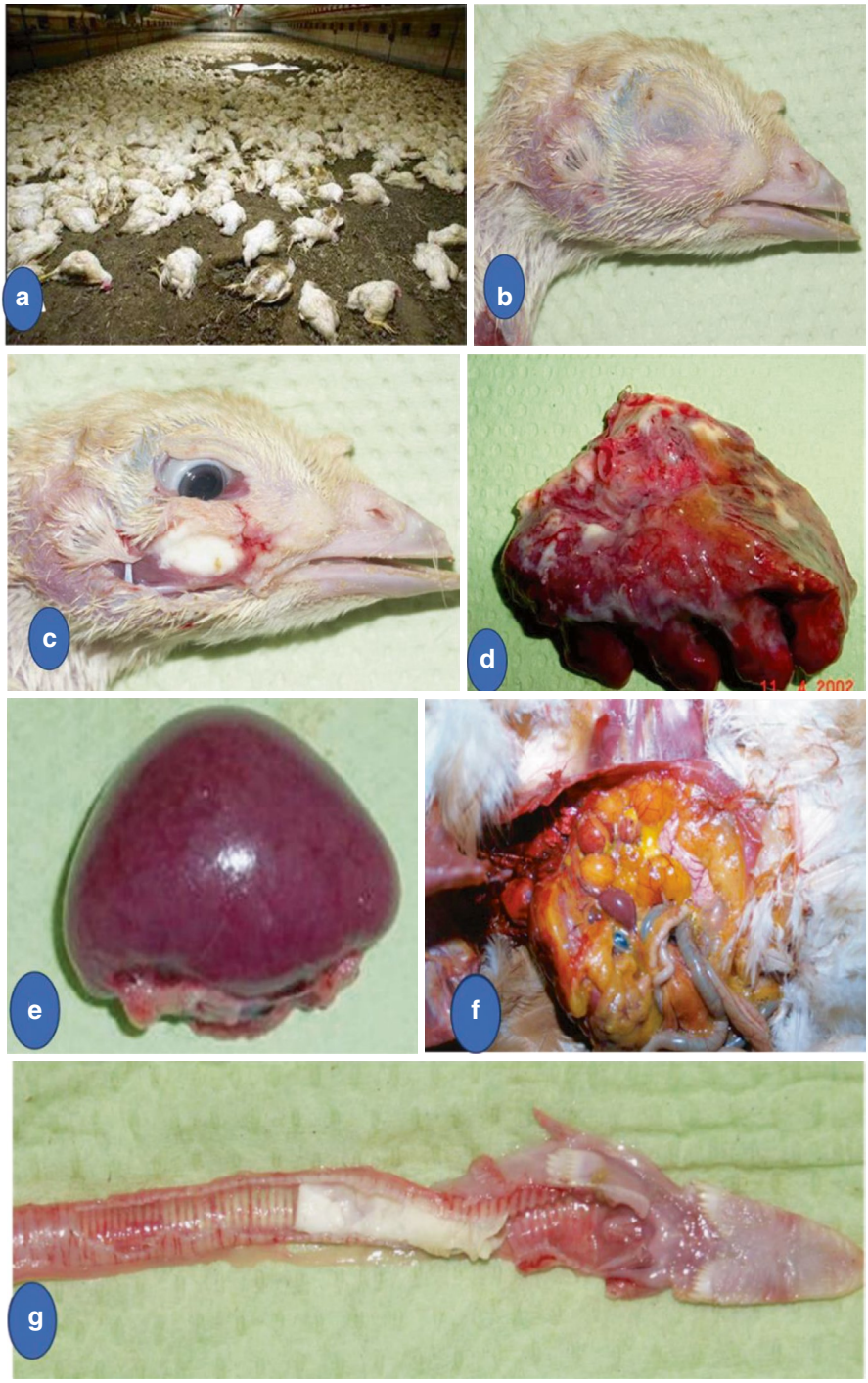

Fig. 1.3 Postmortem lesions of avian influenza in turkeys. (**a**) sudden death, (**b**, **c**) sinusitis, (**d**) pneumonia, (**b**) splenomegaly, (**f**) peritonitis and hemorrhages on the ovarian follicles, (**g**) tracheitis, (**h**) healthy pancreas, (**i**) hemorrhagic pancreatitis (©Hafez, H.M. Fu-Berlin)

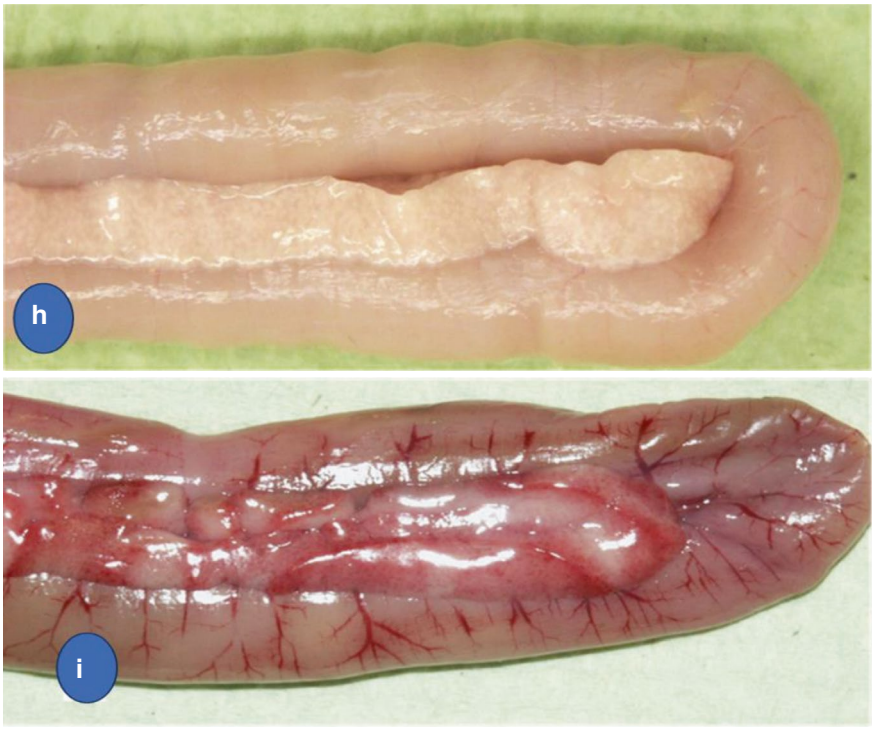

Fig. 1.3 (continued)

the spleen, and inflammation of the pancreas. Blood vessels are usually engorged. Hemorrhage may occur in the trachea, proventriculus, and intestines. HPAI can also cause necrotizing myocarditis. The lining of the gizzard may be easily removed. Young broilers may show signs of severe dehydration with other less pronounced or absent lesions.

1.10 Diagnosis

1.10.1 Sampling

Sampling tracheal swabs from poultry flocks to detect avian influenza viruses is time-consuming and involves a high workload for the staff and a lot of stress for the sampled animals. Swab samples in/on drinkers can easily be taken. When taking water, every animal with a respiratory infection leaves small amounts of its respiratory secretions (mucus) in/on the drinkers. Even before the first clinical signs appear in a flock, the influenza virus is already in small amounts of mucus particles in the drinkers present. By sampling swabs in/on many drinkers in the barn, the smallest amounts of respiratory murus from infected birds in the flock can be detected by PCR technique.

In contrast to the fecal samples, the samples from the drinkers contain only a small amount of DNA and RNA from microorganisms (Sieverding and Hafez 2023).

The PCR results from this field study showed that swabs from the drinkers in the barn yield very reliable and very important information about the presence of avian influenza viruses in a poultry population. However, taking swab samples from many drinkers in the barn is important. Influenza monitoring with swab samples from drinkers is rapid, sensitive, specific, reliable, and inexpensive and can be taken easily by individuals, regardless of the age and the number of birds in the flock. Detecting the avian influenza virus in infected flocks with swab sampling in/on drinkers is an animal-friendly process with an improvement in the statistical significance of the infection status of a flock.

1.10.1.1 Laboratory Diagnosis

Clinical signs and lesions are not pathognomonic. Therefore, isolation, identification, and characterization of the virus involved are essential. The laboratory diagnosis of AI is done in three steps, including (i) identification of influenza type, (ii) identification of subtype, and (iii) pathotyping using animal experiments or sequence analysis of the hemagglutinin cleavage side (Fig. 1.4).

AI virus can be isolated in embryonated chicken eggs by the allantoic sac route or using several cell lines. Depending on the pathotype, the embryos may or may not die within a 5-day observation period, and usually, there are no characteristic lesions to be seen in either the embryo or the allantoic membrane. Hatching eggs inoculated with HPAIV-containing material usually die within 48 h.

The hemagglutination test can detect a hemagglutinating agent in the harvested allantoic fluid; it must be differentiated from other hemagglutinating viruses, such as the Newcastle disease virus and egg drop syndrome. Chicken RBCs are commonly used; however, H1 and H3 influenza viruses isolated from turkeys agglutinate turkey, horse, and guinea pig RBCS may not agglutinate chicken RBCs.

The agar gel immunodiffusion (AGID) test using influenza A antigen can confirm that the isolated virus is influenza A, depending on the matrix and nucleocapsid antigens. PCR can also be used for the detection of the circulating subtype. HI and NI tests can be used for subtyping influenza viruses using specific sera for H5 and H7 subtypes.

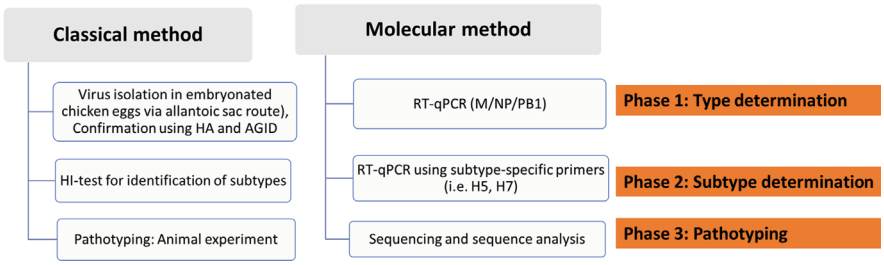

Fig. 1.4 Diagnosis of avian influenza. The laboratory diagnosis of avian influenza is done in three steps. (i) isolation of avian influenza A, (ii) identification of subtype, and (iii) pathotyping using animal experiment or IV: sequence analysis of the hemagglutinin cleavage side

Pathotyping can be determined using the in vivo pathogenicity test and/or sequencing and phylogenetic analysis. In vivo, the pathogenicity test shall be conducted according to the guideline published by the OIE manual by inoculating 4- to 8-week-old specific pathogen-free chicks intravenously. HPAI causes the mortality of 75% of birds within 10 days. Isolates with an IVPI >1.2 are considered HPAI. The cleavage site of HPAI is multiple basic amino acids in the case of HPAI. However, LPAI viruses have monobasic amino acids.

Serological detection. AGID and ELISA can be used to identify antibodies in flocks exposed to infection. AGID detects antibodies toward M1 and nucleocapsid proteins for all type A viruses (group-specific). The AGID test requires large quantities of reagents and takes 24–48 h for results to be obtained, while most commercial ELISAs detect only antibodies toward nucleoproteins. Furthermore, the AGID test may not be suitable as a universal assay for some other species of birds; serum samples from waterfowl do not contain good precipitating antibodies. The HI test is more sensitive and rapid than the AGID test, but it is complicated due to the existence of 16 HA subtypes of AIV.

1.11 Prevention and Control

Since AIVs in nature are maintained in wild aquatic birds, eradicating the infections seems complicated and even impossible. However, every effort should be taken to prevent direct and/or indirect contact between domestic poultry and wild waterfowl as well as vaccination of poultry flocks.

In conjunction with strict quarantine, several vaccination programs have been used to control the disease in commercial turkey flocks. In infected flocks with HPAI, strict quarantine and rapid depopulation of infected flocks remain the only effective methods of stopping AI. The success of vaccination programs depends on the course of the infection, governmental regulations, veterinarians, and the poultry industry. However, using inactivated vaccines against highly pathogenic influenza viruses in some countries has revealed promising results.

1.11.1 Pros and Contras Against the Need for Vaccination

Vaccines against LPAI viruses were successfully used in turkey farms in the United States, demonstrating their potential effectiveness against HPAI viruses (Halvorson 2009). After that, vaccination was implemented in several countries, including Italy (LPAI), Mexico (HPAI), and China (HPAI). In influenza-endemic countries, such as China, Egypt, Indonesia, and Vietnam, vaccination against HPAI has been used after the ineffective implemented stamping out policy. Vaccination of turkeys is commonly used to control HPAI H5N1 infections in many countries (Halvorson 2002; Swayne et al. 2014).

The decision to vaccinate against AI remains challenging (Sims et al. 2016), Fig. 1.5. The outbreaks' severity and economic consequences have led to debates on

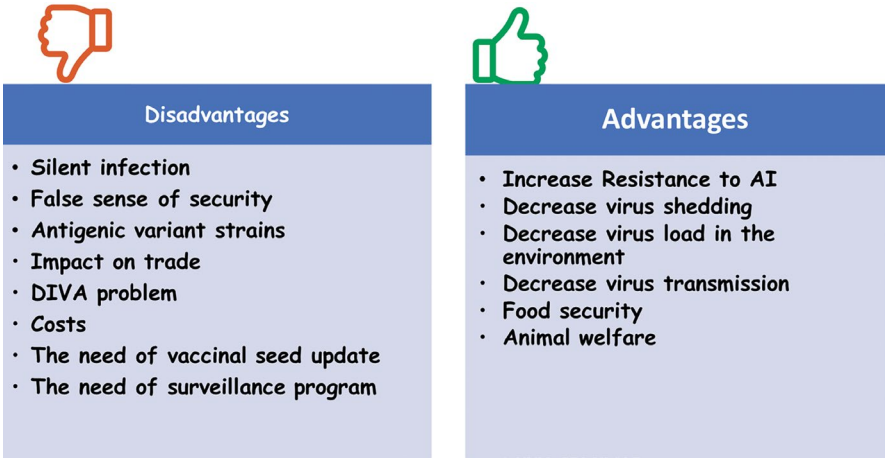

Fig. 1.5 Pros and cons against HPAI influenza vaccines

whether vaccination should be allowed alongside eradicating infected flocks. Poultry farmers advocate for vaccination, citing several reasons: (i) The stamping out method is insufficient in controlling the spread of the deadly AI virus. (ii) The affected regions are too vast to prevent further virus transmission. (iii) Vaccination is vital in safeguarding valuable flocks, especially breeding stock.

However, some scientists argue against vaccination for the following reasons: (i) Vaccination does not completely prevent the infection of flocks and can lead to the development of clinically silent virus carriers. (ii) It can be challenging to distinguish between infected and noninfected birds in vaccinated flocks. (iii) Vaccinating all flocks in affected regions can result in significantly high expenses. (iv) Vaccination may result in additional trade restrictions. (**v**) Virus evolution under vaccination pressure leads to the emergence of antigenically variant strains. In Egypt, since 2006, HPAIV H5N1 of clade 2.2.1 infected a wide range of poultry, including turkeys, and caused severe economic losses. The virus is endemic in poultry in several countries and has diversified into two genetic clades: clade 2.2.1.1 and clade 2.2.1.2. The 2.2.1.1 clade represents immune-escape variants in vaccinated commercial poultry from 2007 to 2011. The 2.2.1.2 clade was detected in humans, nonvaccinated backyards, and commercial poultry (Salaheldin et al. 2017). In 2022, The 2.2.4.4b clade emerged. Generally, it is recommended to vaccinate against influenza with vaccines that provide the Differentiating Infected from Vaccinated Animals (DIVA) principle to detect active infection in vaccinated animals. Capua et al. developed and validated a serological test, "DIVA "(Capua et al. 2003).

Over the years, advancements in vaccine technology and extensive research have proven the safety and effectiveness of vaccines against HPAI. Innovative vaccination strategies, like vector-based vaccines and recombinant technologies, have overcome previous limitations and strengthened confidence in immunization and control measures. The collaborative efforts between international organizations, governments, and research institutions have also played a vital role in establishing

standardized vaccine development, distribution, and administration protocols. These efforts have harmonized global strategies and improved preparedness and response mechanisms for potential HPAI outbreaks. Consumer attitudes have evolved, driven by increased awareness of the public health implications of avian influenza and growing demand for sustainable and ethically produced poultry products. This shift in consumer sentiment has encouraged industry stakeholders to embrace vaccination as a proactive measure to safeguard avian welfare and human health.

In 2023, vaccination against HPAI was approved in several EU countries for several poultry species, such as France (domestic ducks), the Netherlands (layers), Hungary (geese), Italy (turkeys), the Czech Republic (geese), and Belgium.

1.11.1.1 Types of Vaccines

(i) **Inactivated vaccines** prepared in embryonated chicken eggs, used as oil emulsions, and administered intramuscularly or subcutaneously. These vaccines produce humoral immune antibodies.

(ii) **Vectored recombinant** vaccines by inserting the HA gene into the Fowlpox virus vaccine strain **or** herpesvirus turkey (HVT) vectors (Kapczynski et al. 2016).

The host develops an immune response against both influenza and the used vector. Vector vaccines are characterized by the induction of both cell-mediated and humoral immune responses. Also, this type of vaccine can differentiate between vaccinated and infected birds (DIVA), with no antibodies against NP. The disadvantage of these vaccines is that birds exposed to the fowlpox virus will not develop antibodies toward AI (Brugère-Picoux et al. 2015). Several types of H5 vaccines are used, including local and nonlocal field strains and inactivated and recombinant vaccines from historic and recent H5Nx viruses.

Vaccination programs are highly variable; co-infections with viral and bacterial infections are widespread and can negatively influence the vaccine's efficacy. Also, maternal immunity in day-old poults interferes with vaccination at an early age (Abdelwahab and Hafez 2011). A substantial antigenic drift has been reported as an immune evasion mechanism due to mutations in immunogenic epitopes of the hemagglutinin gene 2.2.1.1 (Abdelwahab et al. 2016). Nevertheless, the infection in vaccinated poultry continues (Abdelwahab et al. 2016). However, little is known about HPAI H5N1 infections in vaccinated turkeys (Salaheldin et al. 2017).

1.11.1.2 Vaccination Against Low-Pathogenic Avian Influenza

Generally, the inactivated H9N2 vaccine does not effectively control the transmission of the LPAI virus in poultry (Cui et al. 2021). Inactivated H9N2 vaccines mainly induce humoral immunity, which makes it difficult to interrupt virus infection and shedding in the chicken upper respiratory tract. H9N2 AIV strains are still circulated between vaccinated chickens (Zhong et al. 2014), highlighting the urgent need to develop more effective vaccines that provide cellular and mucosal immunity against H9.

However, there are some trials for developing other vaccine types. Shehata et al. (2020) evaluated the efficacy of H9 plasmid-based DNA targeting the HA gene of H9N2 A/CK/Egypt/SCU8/2014 in turkey poults. The effectiveness of DNA (pVAX-H9 and pCR- H9) vaccine, naked or saponin-adjuvanted, was evaluated in turkey poults at third week of age intramuscularly and challenged 3 weeks later with the same life isolate at a dose level of 10^6 EID_{50}/bird. None of the birds vaccinated with naked or saponin-adjuvanted pVAX-H9 or pCR-H9 showed any clinical signs. However, the pVAX-H9 and pCR-H9 alone did not prevent cloacal and oropharyngeal virus shedding.

On the other hand, saponin-adjuvanted pVAX1-H9 and pCR-H9 prevented cloacal and oropharyngeal virus shedding at the third and fifth days post-challenge, respectively. All vaccinated birds showed high antibody titers in HI (7–8 $\log_2$) in the third week post-vaccination. In conclusion, DNA vaccination with pVAX1-H9 and pCR-H9 could protect turkeys from the H9N2 virus, but vaccination regimes should be improved.

1.11.1.3 Considerations and Essential Components

Several considerations should be considered for preventing and controlling avian influenza (Halvorson 2002, 2009; Swayne et al. 2014; Sims et al. 2016).

1. HPAI should be eradicated from poultry, and vaccination should be used only to deliver eradication. However, stamping out programs are complex, expensive, and labor-intensive. In several countries, many farms are not registered. Passive surveillance systems and farmer compensation schemes should be implemented, particularly if virus elimination is still the immediate goal. Compensation for culled birds is essential to encourage the owner to report the disease as early as possible. It must be paid at or near the actual market value of the birds.
2. Biosecurity is the first line to prevent the introduction and spread of infection. In simple terms, keep pathogens away from poultry and poultry away from pathogens. However, practically, biosecurity can reduce but does not eliminate risks.
3. Vaccination is recommended for countries where there is a risk that stamping out may result in the removal of a major source of food for rural communities and damage the commercial viability of the local poultry industry. If vaccination is to be an option in the control of AI, then it must be used in parallel with enforced biosecurity measures. The objectives and strategies for vaccination should be clear, consistent, and regularly reviewed to align with prevention and control plans.
4. High-quality vaccines that are registered by national authorities should be used if vaccination is implemented. Vaccines should exhibit a good antigenic match to the circulating field strain(s). However, it's important to note that not all virus strains are equally effective in stimulating immunity, which may be attributed to the glycosylation patterns of the HA protein. Therefore, the best approach for evaluating vaccine efficacy is to challenge experiments.

5. The efficacy of the influenza vaccines should be evaluated in the target species. Generally, higher antibodies correlate with efficacy. The potency of vaccines should also be considered. The vaccine should contain 1–5 μg HA/dose or 512–1024 HA unit/dose. Usually, HI titers of >1:32 can prevent mortality, while >1:128 prevent oropharyngeal shedding. The efficacy should be tested in vivo against newly emergent mutants. Turkeys should be vaccinated at least three to four times.
6. The merits of proper vaccination against AI are increasing the resistance of birds to influenza infection and reducing the virus shedding by reducing virus replication in the respiratory and gastrointestinal tracts. However, vaccines cannot prevent infection, virus replication, virus shedding, spread from farm to farm, and problems in diagnosing infected flocks (DIVA strategy).
7. When selecting vaccines, DIVA should be considered. Four strategies can achieve DIVA**:** (i) sentinel birds, where 20 susceptible birds are reared with a vaccinated flock and examined for seroconversion and/or presence of AI antigen; (ii) heterologous neuraminidase strategy, in which neutralization inhibition (NI) is available for all nine NA, but the availability of diagnostics is an issue; and (iii) nonstructural protein I (NS1) DIVA strategy. Theoretically, killed vaccines do not contain NS1, while naturally infected birds develop Ab toward NS1. However, inactivated vaccines are contaminated with NS1 during preparation, so dilution of serum before testing may decrease the nonspecific reactions. (iv) recombinant/subunit vaccine: AGID and ELISA can be used (subunit vaccines lack AI nucleoprotein).
8. Recombinant influenza vaccines using fowlpox or herpesvirus turkeys (HVT) induce both cellular and humoral immune response and provide DIVA strategy (HA-only based vaccines supporting serological DIVA strategies). However, the fowlpox vector vaccine is ineffective in birds exposed previously to fowlpox or birds with immunity to the vector.
9. Vaccination alone without culling affected birds to reduce virus load in the environment will probably not be successful.
10. Continuous updates and development of new diagnostics are crucial in the face of genetic mutations of the virus in endemic countries.
11. Genetic monitoring of the field virus under vaccine pressure is essential. The efficacy of the vaccines should be tested in vivo using the newly emergent mutants. Principally, the closer the amino acid similarity of the vaccine strain, the greater the virus replication and shedding reduction.
12. Particular attention should be given to the household and backyard poultry production systems; the improper disposal of dead birds and wastes is another important factor in disease control failure.
13. Any vaccination policy should include an exit strategy so that countries do not rely on costly long-term vaccination campaigns. It is important to continue using vaccination until infection is controlled and prevented. Vaccination may be required for an extended period in areas where the virus is widespread and elimination is unlikely. Vaccination can be stopped once the disease is under control and the risk of recurrence is low. Rapid detection and management of new outbreaks are crucial to ensure successful control.

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