

The History of Highly-Pathogenic Avian Influenza in Israel (H5-subtypes): from 2006 to 2023

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ABSTRACT

Avian Influenza (AI) has become the largest animal epidemic in the world. So far hundreds of millions birds have died or been culled due to the disease. A novel highly-pathogenic avian influenza (HPAI) subtype H5N1 virus, which emerged in 1996 in domestic geese in China, passed from wild waterfowl as a low-pathogenic (LP) virus, and in the domestic birds was modified to a High Pathogenic (HP) virus due to mutational addition of basic amino acids to the cleavage site of the Hemagglutinin (HA) protein. This "A/goose/Guangdong/1/1996" virus killed poultry but also infected at least 18 people of whom 6 died. In 2003-6 the virus spread to Asia, Europe, Africa and the Middle East. Mass mortality of migratory birds at the Salt Qinghai Lake in West China in 2005 was a turning point in the understanding of the virus co-circulation between poultry and wild birds. Israel is situated on migratory routes for over a billion birds each year in the autumn migration from Europe to Africa and in the northwards spring migration, thus the potential for virus transmission is high. The 1996's parental virus emerged in Israel in 2006, following the Qinghai Lake event, and included meat turkeys, heavy breeders and broilers. Since then, several focal outbreaks occurred in Israel every 1-3 years. In 2012, the same AIV-H5N1 was detected in meat turkeys and found also in alley cats following consumption of the bird carcasses, with 100% identity of the HA gene. A second multi-focal outbreak of AIV-H5N1 appeared in 2015 following the worldwide wave in 2014-15. This outbreak included meat turkeys, heavy breeders and egg layers. These viruses belonged phylogenetically to clade 2.2.1.2, emerged from previous clades 2.2 and 2.2.1.1. Reassortment of viral segments of a descendant-H5 AIV of the Chinese parental virus with AIV-N8 viruses created novel H5N8 viruses, first detected in China in 2010. An 2016's variant of this virus (group-B Gochang-like, clade 2.3.4.4), was discovered in May-June 2016 in wild swans at Lake Ubsu-Nur on the Russian-Mongolian border, and reached Central Asia, Europe, Africa, Middle East and Israel. The Ubsu-Nur 2016's AIV-H5N8 was genetically identical to an AIV-H5N8 that emerged in Israel six months later during the autumn migration. This was the first H5N8 outbreak in Israel, which included meat and breeder turkeys, heavy and light breeders, layers, breeder ducks and backyard poultry, but also many wild birds, not seen in the previous H5N1 outbreaks, of at least 16 species mostly waterfowl and raptors. The cleavage site of HA gene showed similarity between poultry and wild birds, as well as to grp B-2.3.4.4 European-Asian H5N8 viruses. Since October 2020, new H5 reassortants with LPAI viruses from wild birds contributed the Neuraminidase (NA) glycoprotein, have appeared in Europe and created new H5 strains. A second global outbreak of a new H5 variant occurred in 2020-21 in Russia and the Palearctic region. Following this global outbreak, a second AIV-H5N8 outbreak appeared in Israel in 2020 during the autumn migration. The outbreak included meat and breeder turkeys, heavy breeders, broilers, game birds mainly black swans (*Cygnus atratus*), and wild birds. The 2021-22's AIV-H5N1 epidemic season was the largest so far in Europe and Eurasia, with about 2500 outbreaks in poultry and culling of 50 million birds, and about 3600 detections in wild birds. That outbreak followed the 2020's wave of the new AIV-H5N8.

Following this epidemic, a third multi-focal outbreak of AIV-H5N1 occurred in Israel in 2021, in meat and breeder turkeys, heavy breeders, organic egg layers and meat ducks. The 2021's outbreak was characterized also by affecting many wild birds, not seen before in H5N1's outbreaks in Israel, while the largest affected population was Common cranes (*Grus grus*) on their migration routes from Russia and Scandinavia to Ethiopia and Sudan through Israel, undergoing mass mortality of almost 10,000 birds. Other threatened species like Marbled teal (*Marmaronetta angustirostris*) have died also due to this virus, as well as hundreds of Great white pelicans (*Pelecanus onocrotalus*), and several species of waterfowl and raptors. Classification of the viruses by whole genome sequencing (WGS) revealed the same group B-2.3.4.4 clade as in the H5N8 events and similarity between poultry and wild birds. This virus probably came from Russia in the autumn migration. A new outbreak of AIV-H5N1 began emerging in Israel from November 2022, in meat turkeys, heavy and light breeders, broilers. Unlike the 2021's outbreak, this virus affected only a few wild birds. HA gene sequencing revealed the virus belonged to the same group B-2.3.4.4 clade as in previous outbreaks. In conclusion, all avian influenza subtype H5 outbreaks in Israel followed the European-Eurasian outbreaks. The virus origin was from South East Asia, reaching Israel through spillover with migrating birds via Eurasia and Europe.

Keywords: Avian Influenza; AIV-H5N1; AIV-H5N8; Clade; Spillover.

INTRODUCTION

Avian Influenza has become the largest animal epidemic in the world, until today hundreds of millions of birds have died or been culled due this infection.

Avian Influenza Virus (AIV) is an 8-segmented *Orthomyxoviridae* type A enveloped single-stranded RNA virus with a size ranging from 80 to 120 nm. The virus contains two important envelop glycoproteins that determine its classification into subtypes, one of 18 different Haemagglutinin (HA) proteins and one of 11 different Neuraminidase (NA) proteins. However birds have one of 16 different HA's and one of 9 different NA's. Wild birds are natural reservoir for the 16 HA and the 9 NA subtypes of AIV. The HA protein must undergo proteolytic cleavage into two subunits to allow conformational changes required for membrane fusion and subsequently the virus pathogenesis process. According the number of basic amino acids (lysine, arginine) in its cleavage site (a part of the HA protein) the virus may be defined as low-pathogenic (LP, 1 or 2 basic amino acids) or high-pathogenic (HP, more than 2 amino acids). Only H5 and H7 AIV's are defined as highly-virulent viruses. AIV-HPAI's

originated in China as a mutation from AIV-LPAI's that were introduced from wild birds to farm birds – and multiplied in the latter, mainly due to RNA-polymerase errors that caused the addition of basic amino acids to the cleavage site or the replacement of non-basic amino acids with basic ones, but also changes in other genes (1, 2).

The most quoted date for the beginning of Avian Influenza (AI) in poultry is 1878 when a sudden plague-like disease in poultry appeared in Italy, initially known as "fowl plague" confused with acute septicemic fowl cholera but later renamed to "highly pathogenic avian influenza", given by the Italian veterinarian Edoardo Perroncito (3). A defined AIV-H5N1 virus was isolated for the first time in 1959 from chickens in Scotland (4), AIV-H5N3 in 1961 from common terns in South Africa (5), and a list of many other HPAI viruses from poultry, including H5 and H7 subtypes (6). All these were sporadic outbreaks in commercial or backyard poultry and non-pathogenic for humans. Outbreaks of HPAI's viruses until 1995 caused only minimal losses (7), while the eruption of the H5 outbreaks led to the current waves which began in 1996.

Highly-pathogenic H5N1 emerged in 1996 as a novel strain in domestic geese in Guangdong, Southern China, that passed from wild waterfowl, while in the domestic birds the virus was modified by a mutation to become a highly-pathogenic virus (by addition of basic amino acids

DEDICATION:

This article is dedicated in memory of Prof. Yorham Weisman, Dr. Shimon Perk and Dr. Ezra Rosenbluth, all of whom mentioned played an important role in the research concerning Avian Influenza.

to the cleavage site). This novel virus killed in 1997 over 3 million chickens in Hong Kong, and then, for the first time in humans, infected at least 18 people in Hong Kong, 6 of them died (the epidemic in humans was called "Hong Kong Influenza") (8). Therefore, all bird populations on the island were culled to disable the spread of the virus. This progenitor virus was called "A/goose/Guangdong/1/1996" (9).

In 2003-6 the virus spread in Asia, Europe, Africa and the Middle East, and burst into the public mind as dangerous to birds and to humans. The turning point happened in 2005 when thousands of migratory birds died due to HPAI at one of the main migration stations in western China, the salt lake called "Qinghai Lake". Mass mortality of migratory birds at the salt Qinghai Lake in West China in 2005 was a turning point in the understanding of the virus co-circulation between poultry and wild birds (10).

Since 2003 until 6 January 2023, WHO reported 868 cases of human infection by AIV-H5N1 from 21 countries of which 457 individuals died (Case Fatality Rate, CFR of 53%) (11), with another two cases reported in February 2023 in Cambodia, one of them was fatal (12). Transmission between countries has been largely attributed to migratory wild birds. Most of the human deaths were in Egypt, Indonesia and Vietnam (11).

In the last two years, 2021-22, AIV affected most European countries and is considered there endemic. Israel is situated on migratory routes of more than 500 million birds twice a year, in the autumn migration from Europe to Africa and in the spring migration northwards (13). As most of the migratory birds arrive from AIV-infected countries, and some stay to winter in Israel, the potential for transmission of the virus from wild birds in Israel is high.

The HP AIV-H5N1 virus has a short incubation period in the affected birds, with a huge swift mortality within 24-48 hours, leading to very high economical losses. The virus may affect all types of domestic and captive birds, although wild waterfowl may serve as subclinical carriers (1). The characteristic clinical signs in commercial poultry include sudden onset of severe illness along with rapid mortality, up to 100% in a few days, even 1-2 days, without apparent signs. When signs do appear they include depression, ruffled feathers, severe respiratory distress, coughing, sneezing, eye inflammation or conjunctivitis, sinusitis, cyanosis of the head, combs and wattles, swelling/edema of the head, face and sinuses,

diarrhea, nervous signs, and in breeders and layers a drop or cessation of egg production (1).

According reports from 2005 onwards, the virus may be also fatal and kill these subclinical carrier birds (14, 15), however sporadic deaths of wild waterfowl and other migratory birds due to ancient AI viruses were reported even years before (6). The virus is spread by respiration, nasal and mouth discharge, droppings or close contact with infected birds. Humans can also be infected through bird slaughter, feather plucking, cleaning and preparation of poultry meat for cooking, eating raw meat and contact with contaminated blood, for example by sucking contaminated blood of poultry as customary in eastern countries like Japan, Thailand, etc (16). Elevated concern of mutations by gene transfer between influenza viruses, changes in receptor affinity (antigenic shift), and the emergence of a novel pandemic virus that has the feature of man-to-man infection always exists.

Post-mortem lesions of HP AIV's include mainly hemorrhages, edema and necrosis of multiple organs along with inflammation, including skin of the comb or wattles, legs and toes. Most severely affected organs are liver, pancreas, heart, lungs, kidneys and brain. Due to the hyper-acute nature of the disease, it is possible that none of the mentioned lesions will be seen (1).

Another highly-pathogenic reassortment of H5 with HxN8 viruses, namely H5N8, was first detected in a live poultry market in China in 2010, then in South Korea and Japan. The hemagglutinin coding segment of H5N8 was a descendant of the Chinese parent virus – 1996 HPAI H5N1 (A/goose/Guangdong/1/1996).

In fact, the first known outbreak of AIV-H5N8 subtype occurred in poultry in Ireland in 1983 (A/duck/Ireland/113/1983, A/turkey/Ireland/1378/1983), over 300,000 domestic ducks, chickens and turkeys were culled where turkeys were the most susceptible bird (6). Twenty years later the virus was reported in US (A/avian/New York/Sg-00418/2003). These viruses were different from the current 21st century viruses.

A 2014 variant of H5N8 appeared for the first time in South Korea as an outbreak in breeding ducks, other poultry and wild birds. The variant reached China, Japan, Europe and North America (group-A Buan-like, clade 2.3.4.4), but did not reach Israel (17, 18, 19). The virus was introduced into Europe probably via the autumn migration of wild birds returning from Russia and Asia with routes going from

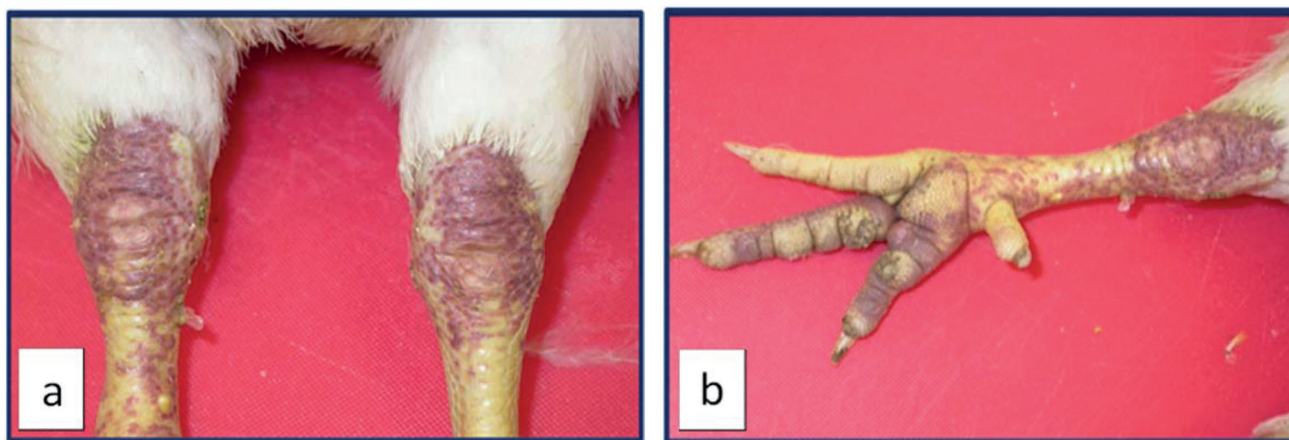


Figure 1. Focal hemorrhages in legs (a) and toes (b) skin of heavy breeders infected with AIV-H5N1 (Ein Shemer, 2010, photos by Dr. A. Lublin, Kimron Veterinary Institute, Bet Dagan).

northeast to southwest. However, a 2016's variant of this virus (group-B Gochang-like), reported in South Korea and Taiwan in domestic and wild birds, discovered in wild swans in Lake Ubsu-Nur in Tyva Republic on the border of Russia-Mongolia in May-June 2016, reached Central Asia, Europe and the Middle East including Israel and Africa. Group-B variant belongs to the same 2.3.4.4 clade. Clade 2.3.4.4 was found to reassort with 6 different neuraminidase genes. As different H5Nx viruses may circulate at the same time in the same area, the contribution of the migratory birds was clear.

Clinical signs in birds of this variant virus include apathy, incoordination, seizures, recumbency and mortality. Unlike AIV-H5N1, AIV-H5N8 variants it is less virulent to humans. A virus of the H5N8 strain caused mild and limited or asymptomatic disease in seven poultry workers in south of Russia in an outbreak of AIV-H5N8 on a poultry farm (February 2021), without person-to-person transmission (20).

THE HISTORY OF VIRULENT AVIAN INFLUENZA VIRUSES IN ISRAEL.

Before the AIV-H5N1 eruption.

Virulent AIV viruses in Israel are known since the 70-80's of the previous century concurrently in domestic and wild birds. They include outbreaks of AIV-H7N2 (HP) in chickens (broilers) in Degania presenting as a respiratory disease, in breeder turkeys in Ramon and in duck farms. Concurrently, AIV disease was found in feral mallards, rock partridges and starlings (21, 22, 23, 24, 25, 26). In the turkey farms, the

AIV infection occurred about 2 months after mortality of about 200 wild mallards that were found in close proximity to the turkey premises. Up to 80% mortality was found in the turkeys and a sharp drop in egg laying in the chickens, from 50-70% to 5-10%. The diagnostic tools at that time were serology and virus isolation (27, 28). The first isolation of avian influenza virus in Israel was in migratory starlings (*Sturnus vulgaris*) however it was a non-pathogenic (NP) virus (subtype H7N7) (21, 29).

AIV-H5 WAVES OF OUTBREAKS IN ISRAEL.

2006's multi-focal outbreak (H5N1). The 1996's Guangdong virus appeared in Israel for the first time in March 2006, following the Chinese Qinghai Lake event, and was identical to Egyptian strains. The first reported case of AIV-H5N1 in the outbreak in the Gaza Strip was 5 days later, and in Jordan, 7 days later. The first reported case of HPAI-H5N1 in Egypt was one month before the initiation of the outbreak in Israel. This outbreak included nine events in the Gaza Envelope area and in the Jerusalem area: six meat turkey farms, two heavy breeder farms and one broiler farm, and lasted 2 weeks. The maximal daily mortality was 35% (30, 31).

2008-2012's spot-separated outbreaks (H5N1). Since the 2006's multi-focal outbreak of AIV-H5N1, several focal distinct outbreaks occurred in Israel averagely every 1-3 years. These included: a back-yard chicken in 2008, heavy breeders in 2010 (focal hemorrhages in legs and toes skin are presented in Figure 1), and an Emu in a petting zoo, 3 flocks of meat turkeys in 2011 and one of them in the



Figure 2. A cat infected by AIV-H5N1 after consumption of carcasses of turkeys infected by this virus, displaying eye inflammation (photo by the courtesy of Dr. M. Bellaich, Kimron Veterinary Institute, Bet Dagan).

Palestinian Authority. In addition, in 2011 a Marsh Harrier (*Circus aeruginosus*) that was found ill in Jordan Valley and died later was found positive for AIV-H5N1 presence.

In 2012, two meat turkey flocks were found positive to AIV-H5N1. An outbreak of the same H5N1 virus was detected in alley cats following consumption of infected turkey carcasses from one of the flocks (32). The cats were seen eating sections of carcasses of the dead or euthanized turkeys. Clinical signs in the cats occurred several days later, mainly respiratory, dyspnea, ocular or nasal discharge and weakness, up to their acute death. The nucleotide sequences of HA genes of viruses isolated from the turkeys (ty/Israel/289/2012) and from the cats (cat/Israel/387/2012) showed 100% similarity, with 1.7% difference from the other turkey flock examined. One of these cats is presented in Figure 2.

2015's multi-focal outbreak (H5N1). The second multi-focal outbreak of AIV-H5N1 occurred in January 2015 following a worldwide wave of outbreaks in 2014–15. This outbreak lasted almost one month, in eight flocks, six meat turkey flocks and two heavy breeder flocks, all from the North-Ha'Sharon region. A Spur-winged Lapwing (*Vanellus spinosus*) found pecking at the manure of a turkey flock which had been euthanized. The Spur-winged Lapwing bird presented

with clinical signs of AIV and died, and was diagnosed with AIV-H5N1. This outbreak included also four flocks from the Palestinian Authority, one of meat turkeys and three egg layers, from Jenin, Qalqiliya and Jerusalem districts. These flocks extended the outbreak period by another 6 weeks. The Laboratory of Avian Diseases at Kimron Veterinary Institute, Bet Dagan, assisted in testing some of the samples from the Gaza Strip, found 55 positive farms (most of the tested farms), most of them ducks and egg layers. Another focal event occurred 4 months later (in May 2015) in two meat turkey farms in the North of Israel, on the Lebanese border (33).

The AIV-H5N1 isolates belonged phylogenetically to clade 2.2.1.2 according to WHO nomenclature, emerging from previous clades, 2.2 in 2006's outbreak and 2.2.1 and 2.2.1.1.a in 2010–2012's focal outbreaks.

Figure 3 presents the full HA nucleotide sequences of AIV-H5N1 outbreaks – 2006, 2008–2012 (intermediate period between multi-focal outbreaks) and 2015, compared to global (mainly Egyptian) isolates. There is less than 0.5% difference in the sequences of the various isolates within 2015's outbreak, 3–4% difference between 2015's and 2008–2012's viruses, and about 7% difference between 2015's and 2006's outbreak (which resembles the sequence of the Chinese progenitor virus). As seen in Figure 3, some of the viruses of poultry in Israel are phylogenetically identical to viruses from ducks and chickens from Egypt and the Gaza Strip and the others are almost identical.

2016's multi-focal outbreak (H5N8). The first AIV-H5N8 virus appeared in Israel in November 2016 and lasted until February 2017. This outbreak in commercial birds included mainly meat turkeys but also breeder turkeys, heavy and light breeders, egg layers, breeder ducks and backyard poultry. In addition, many wild birds, not seen in the previous H5N1 outbreaks were affected. The affected wild birds included at least 16 species from 8 orders, mostly waterfowl and other water-inhabiting birds, and birds of prey (34).

The AIV-H5N8 diagnosed in late May–early June 2016 in wild birds in the Ubsu-Nur Lake, was genetically very similar to the virus that appeared in Israel six months later during the autumn migration to Africa, passing through Israel. Analysis of nucleotide sequences of the cleavage site of HA gene showed similarity of the isolates among them-

2015

2008-2012

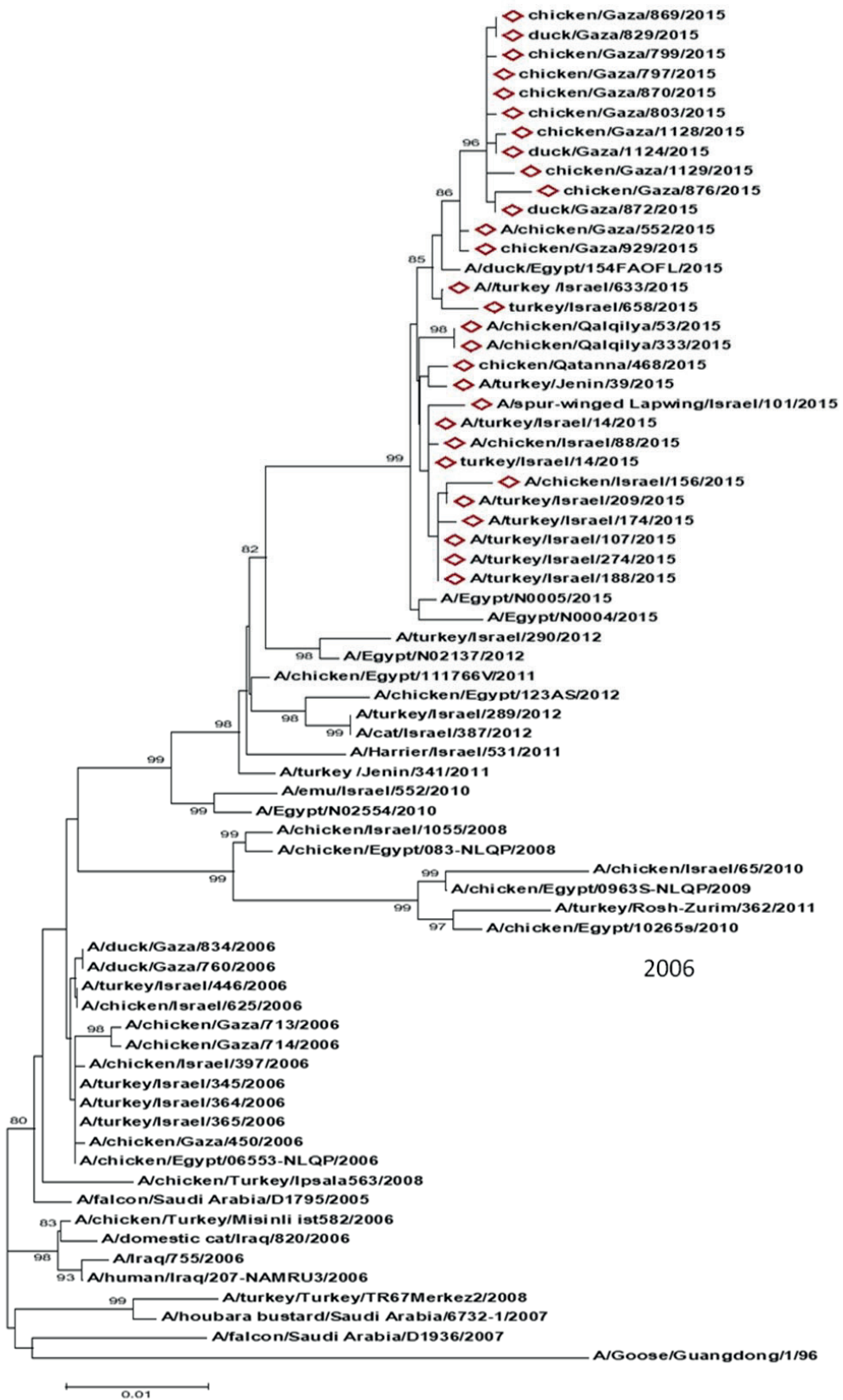


Figure 3. Full HA nucleotide sequences of AIV-H5N1 outbreaks – 2006, 2008-2012 (intermediate period between multi-focal outbreaks) and 2015, compared to global isolates.



Figure 4. HA-2 moiety nucleotide sequences of 2016's AIV-H5N8 outbreak compared to global isolates (Israel's viruses are presented in blue color).



Figure 5. Black swan (*Cygnus atratus*).

selves, with very minor differences, and similarity to H5N8 viruses from other parts of the world (in Asia and Europe), and belonging to clade 2.3.4.4 group B as these viruses (see Figure 4). A single case of AIV-H5N8 positive bird was discovered in January 2018 in a Eurasian eagle-owl (*Bubo bubo*) in Jerusalem.

Figure 4 presents HA-2 moiety (~900 bp out of HA total ~1800 bp) nucleotide sequences of 2016's AIV-H5N8 outbreak compared to global isolates. The Israel viruses belong to clade 2.3.4.4 group B (Gochang-like) as the Chinese and the European viruses, but were different from the clade 2.3.4.4 group A (Buan-like) of 2014's outbreak that had not reached Israel. As seen in the figure, the differences from the global viruses are minor but a sub-group of the Israel viruses was almost 2% different from most of the viruses.

2020's multi-focal outbreak (H5N8). Another global outbreak of a new H5 variant appeared in 2020-2021 in a farm and in wild birds in Russia and the entire Palearctic region. Since October 2020, new H5Nx reassortants have appeared in Europe on the basis of AIV-H5N8 clade 2.3.4.4B that contributed the HA glycoprotein, while LPAI viruses from wild birds in Asia and Europe contributed the NA glycoprotein, thus creating new strains of H5N1, H5N2, H5N3, H5N4, H5N5 and H5N8 (35).

A second AIV-H5N8 outbreak in Israel appeared in October 2020 during the autumn migration season, following the outbreak worldwide. Few months before, in the summer of 2020, there was an outbreak in Hungary, a country on the axis of crane migration from Scandinavia to Israel, also in

Russia from which another migration route originates. This outbreak in Israel lasted for 3 months. The affected poultry branches were meat turkeys, breeder turkeys, heavy breeders and broilers.

This outbreak included game birds in several zoological parks and zoos mainly black swans (*Cygnus atratus*) (Figure 5) but also few a wild birds (Great white pelican, Eurasian eagle owl, Northern shoveler).

2021's multi-focal outbreak (H5N1).

From late 2021 to 2022, a new H5N1 virus causing poultry outbreaks worldwide belonging to clade 2.3.4.4B with a wild bird adapted N1 gene emerged. Those Clade 2.3.4.4B AIV-H5N1 European and Eurasian viruses followed the 2020 wave of a new H5N8 that contributed the hemagglutinin H5 to several reassortants with NA, including N1. The new AIV-H5N1 viruses became predominant in Asia, Africa, Europe, and the Middle East by the end of 2021 (36, 37).

The 2021–2022 the highly pathogenic avian influenza (HPAI) epidemic season was the largest HPAI epidemic so far observed in Europe. There was a total of about 2500 outbreaks in poultry with more than 50 million birds culled in the affected establishments. About 200 outbreaks were identified in captive birds. About 3600 HPAI virus detections were made in wild birds with an unprecedented geographical reach extent from Svalbard Islands to South Portugal and Ukraine, affecting 37 European countries (38).

Following the large HPAI epidemic in Europe, a third multi-focal outbreak of AIV-H5N1 occurred in October



Figure 6. Common cranes (*Grus grus*).

2021, first in a meat turkey farm in Northern Israel. The 2021's outbreak lasted until February 2022 and included over 10 meat turkey farms, one farm of turkey breeders, three heavy breeder farms, several farms of organic egg layers and a meat duck farm. The biggest outbreak was in an aggregate of over 50 premises of egg layers in a Moshav from North Israel, supplying almost 10% of the monthly supply of table eggs. Unfortunately, over 550,000 layers were culled. At least 85% of the affected poultry farms were close to large bodies of water with abundant wild birds, especially waterfowl.

The 2021's outbreak was characterized by a large number of wild birds, not seen before in AIV-H5N1's outbreaks in Israel, while the largest population was that of Common crane (*Grus grus*) (Figure 6) in their migration route from Russia and Scandinavia to North-East Africa, Ethiopia and Sudan, through Israel. The affected crane population underwent mass mortality of almost 10,000 birds in Agamon Hula, a part of the Hula Valley, a large water lake on the route of Jordan River in North-East Israel, but also in several other locations. Action was focused on disposing the carcasses, monitoring the spread of the outbreak, and reducing the large amounts of food (grains) spread to the cranes to minimize their damage to the fish raised in nearby ponds. Also, globally threatened species such as Marbled teal (*Marmaronetta angustirostris*) died, as have hundreds of Great white pelicans (*Pelecanus onocrotalus*) in several locations in Israel especially water reservoirs. Pelicans migrate from Russia to East and Central Africa while passing through Israel, however some of these birds stay to winter in

Israel. Several other wild birds submitted to the laboratory of Avian Diseases at KVI and were found H5N1-positive, these included several species of waterfowl, several of raptors, and a hooded crow.

A genomic classification by whole genome sequencing (WGS) of about 45 isolates of poultry and wild birds (carried out by Prof. Michal Mandelboim and Dr. Neta Zuckerman, the Central Virology Laboratory, Sheba Medical Centre, Ramat-Gan), revealed in all of them the 2.3.4.4B clade, the same hemagglutinin as in the H5N8 events but different from the previous 2015's H5N1 outbreak (2.2.1). It was not possible to differentiate between the genomes of poultry and those of wild birds' viruses.

Figure 7 presents a HA-Phylogenetic tree of 2020-2021's AIV-H5N1 and AIV-H5N8 outbreaks, 2016's AIV-H5N8 (Israeli and global isolates), and another global AIV subtype (H5N6). As seen in the figure, the sequence of the first affected poultry farm in Israel (brown-colored) is identical to those of poultry and wild birds from Russia and Italy (marked in the figure). The H5N8 Israeli isolates are green-colored. The difference between the two H5N8's Israel outbreaks reached 1-2%.

Figure 8 displays pancreatic hemorrhages in a Common crane (*Grus grus*) affected with AIV-H5N1 (A) and in a Black swan (*Cygnus atratus*) affected with AIV-H5N8 (B). Pancreatic and splenic necrosis were reported as a common pathological findings in both Galliformes and Anseriformes infected by AIV-H5N1 in the United Kingdom (39). The pathological presentation of the lesions in both birds are similar.

Poultry and wild birds from
Russia and Italy

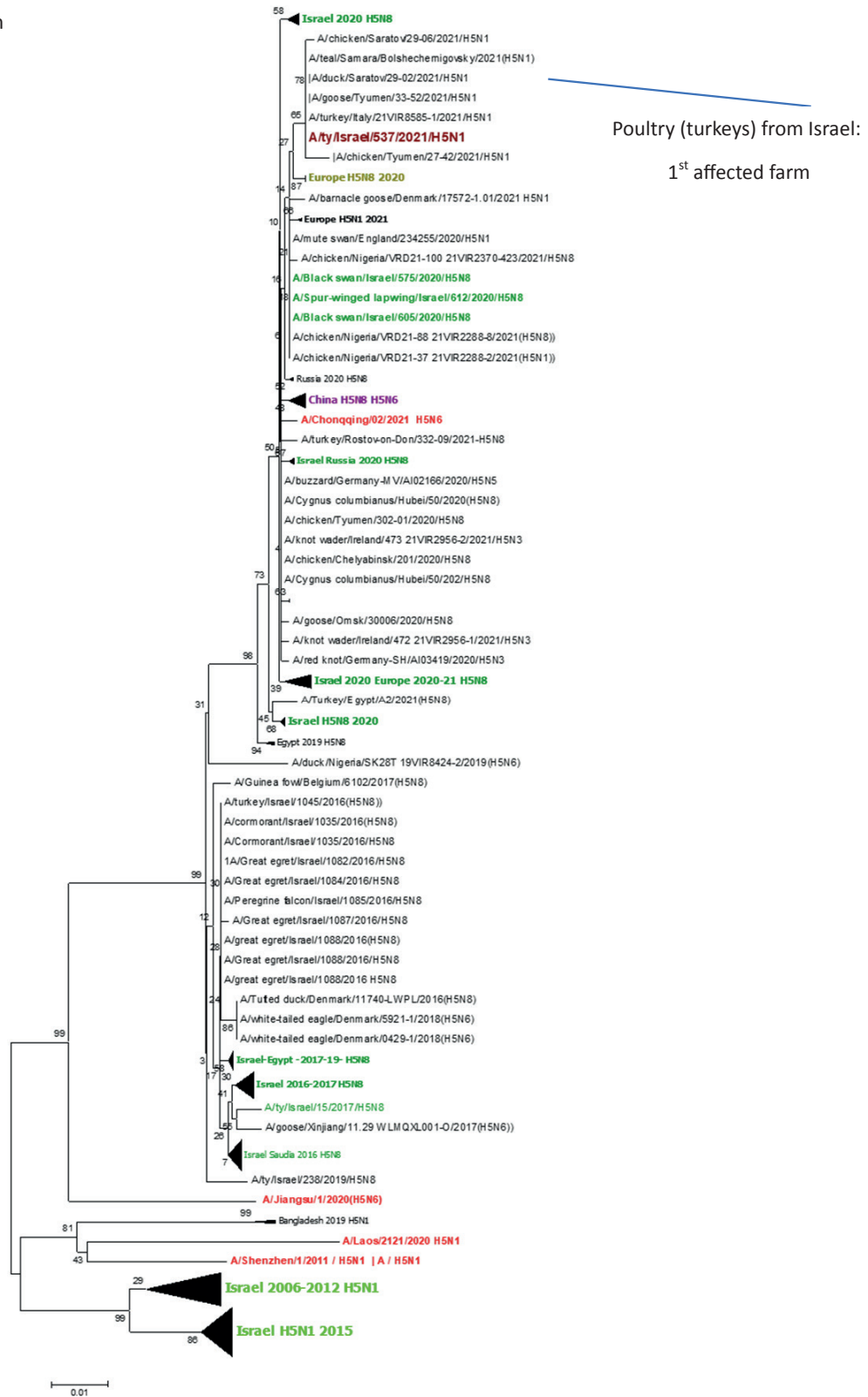


Figure 7. HA-Phylogenetic tree of 2021's H5N1-AIV and 2020-2021's H5N8-AIV outbreaks (Israel H5N1 virus is brown-colored, and H5N8 viruses are green-colored), 2016's H5N8-AIV (Israeli and global isolates, Israeli isolates are green-colored), and other global AIV subtypes (H5N1, H5N6, red-colored).

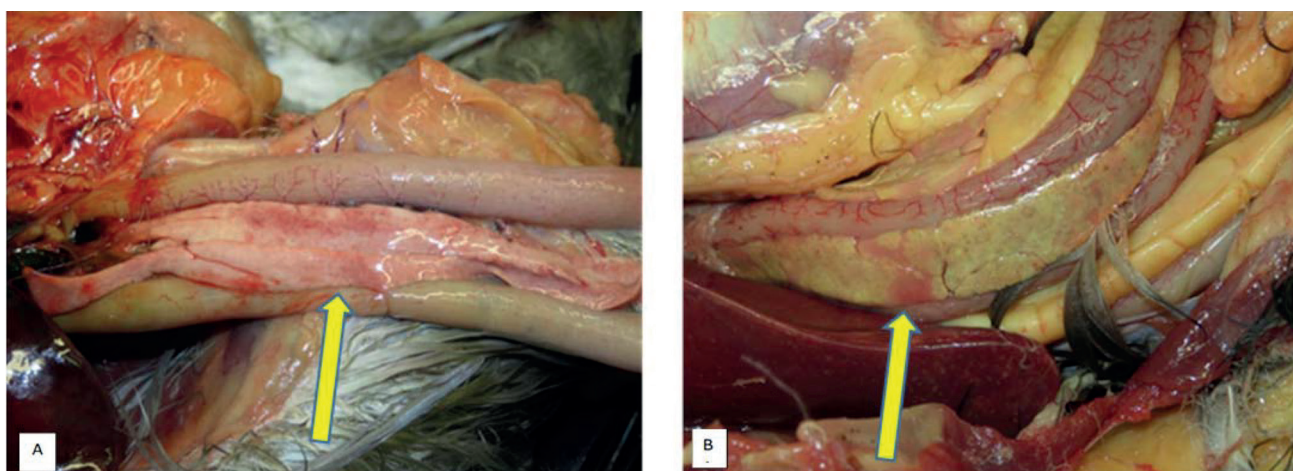


Figure 8. Pancreatic hemorrhages (marked by arrows) in a Common crane (*Grus grus*) affected with AIV-H5N1 (A) and in a Black swan (*Cygnus atratus*) affected with AIV-H5N8 (B) (photos by Dr. A. Berkowitz, Kimron Veterinary Institute, Bet Dagan).

2022's multi-focal outbreak (H5N1). According to all predictions based on the H5N1 outbreak in Europe and the migratory birds suspected of carrying the virus to Israel, a new outbreak of AIV-H5N1 indeed appeared in Israel since November 2022, starting in a meat turkeys flock, and lasted until January 2023. The outbreak included six meat turkey farms, two heavy breeder farms, one light breeder farm and two broiler farms. The appearance of AIV-H5N1 in broiler farms is uncommon and has almost never occurred in Israel in the past, possibly because of the young age of those birds. Unlike in the previous outbreaks, only three wild birds out of many which were tested, were found positive for AIV-H5N1.

DISCUSSION AND SUMMARY

Since the original A/goose/Guangdong/1996 H5N1 virus the HPAI-subtype H5N1 virus was detected and isolated from domestic poultry as well as wild birds in Asia, Europe and Africa, causing sporadic infections in humans and raised pandemic concerns. The virus is continuously co-circulating between domestic and wild birds and presents remarkable genetic diversity due to accumulating point mutations since the low pathogenicity AI viruses of H5 subtype were introduced into poultry. Initially, the importance of birds in the transmission of the virus between continents and countries was doubtful, but over time it has become clear that they are a very significant factor. It is accepted that both wild birds and domestic poultry participate in mutual transitions of AI viruses and their spread between continents and countries and within countries (7).

So far, AIV virus-H5 types have affected 66 countries, causing loss of hundreds of millions of birds (40). The virus entered into Israel through migratory birds and has spread to commercial poultry houses of all poultry sectors. Subtypes H5N1 and H5N8 of AI viruses replace each other along the timeline through common HA-5 glycoprotein and reassortment with other strains that provide the NA glycoprotein. All outbreaks in Israel emerged following Eurasian outbreaks and originated from these viruses and therefore the viruses isolated in Israel are similar or even identical to the European viruses.

Israel has experienced six AIV-H5-subtypes outbreaks (H5N1 and H5N8) in the years 2006-2023. All the outbreaks in Israel appeared following outbreaks in Eurasia and Europe. The first 2006's outbreak appeared after the Chinese Qinghai Lake event in 2005 (10). In that outbreak very few infected migratory birds were detected out of thousands tested and it can be assumed that after the initial entry of the virus, it underwent a secondary spread to various poultry farms in Israel probably through the movement of people and vehicles that may have transferred contaminated feces from infected to susceptible birds. According Balicer *et al* (41), several epidemiologic links between outbreak foci were identified, they and the near-simultaneous detection of several outbreak foci, increase the likelihood that the virus disseminated through use of shared vehicles or by personnel. Still, they did not rule out involvement of migratory birds in disease transmission. As can be seen in Figure 3, the viruses of poultry in Israel are identical or almost identical to viruses

from ducks and chickens from Egypt and Gaza Strip. The Egyptian researchers reported on outbreaks in commercial and domestic poultry in Egypt since February 2006 (42), one month before the first case in Israel in March 2006. The first affected farms in Israel were in the Gaza Envelope, leading to the assumption that the outbreak began following the introduction of the virus from the Gaza Strip.

Since 2014, HPAI clade 2.3.4.4 viruses have spread rapidly via migratory wild aquatic birds and have evolved through reassortment with prevailing local LPAI viruses (43). The 2015's outbreak appeared following outbreaks in Europe in 2014-15. In this outbreak similarly, only very few wild birds were found infected with the AI virus. The 2015's outbreak viruses were found to be also very close to viruses from ducks and chickens in Gaza strip and Egypt, and the sequences of HA were dissimilar from the 2006's AI viruses by 3-4% (Figure 3).

The 2016's AIV-H5N8 isolates are almost identical to European viruses such as from Poland, France and Croatia (Figure 4), and followed the global evolution of the virus, that is the appearance of new viruses such as H5N8 through reassortment. This was the first outbreak in Israel in which many wild birds were found to be infected with the virus. The similarity of the sequences of wild birds as well as domestic birds to sequences of European birds (Figure 4) is proof that the AI viruses were introduced from Europe through the seasonal migration of birds.

Since October 2020, new H5Nx reassortants have appeared in Europe on the basis of AIV-H5N8 clade 2.3.4.4B (34). Following outbreaks in Russia and other European countries in the summer of 2020, a second wave of AIV-H5N8 appeared in Israel several months later in the autumn migration of wild birds to Israel. The HA sequences of the birds in Israel were similar to those of the European and Russian viruses, as can be seen in Figure 7.

Comparing the AIV-H5N1 sequences of the 2021's outbreak in Israel, following the global 2020-21's outbreak (38, 44) to those of Europe, for example, poultry in Israel to poultry and wild birds in Italy and Russia as can be seen in Figure 7, is evidence to the introduction of the virus from Europe to Israel in the autumn migration. According sequences comparison carried out in the Laboratory of Avian Diseases at KVI, same H5N1 virus found about six months before in Africa, probably returned to Europe and Russia in the spring migration before its introduction to Israel in

the successive 2021's autumn migration. As can be seen in Figures 4 and 7, since the 2016's outbreak in Israel, domestic and wild birds have almost the same H5 HA sequences. In those outbreaks, many wild birds, some of which were positive for AIV-H5, were found in close proximity to poultry that were also positive for AIV-H5. These findings may be an indication of the importance of wild birds in the transmission of the virus to commercial poultry in different locations in Israel.

The most severely affected bird population in Israel at this outbreak (global 2020-21's outbreak) was that of Common cranes (*Grus grus*) in their migration route from Russia and Scandinavia to North-East Africa countries through Israel (45). These big birds underwent mass mortality of almost 10,000 individuals in Agamon Hula National Park, one of the largest gathering at the route of their migration to Africa. Some of the birds remain in Israel for the winter, while most of them fly to Ethiopia, Sudan or farther as their final wintering destinations. Combat against further spread of the virus was focused on disposing the carcasses, monitoring the spread of the outbreak, monitoring presence of viral particles in water, and reducing the large amounts of food (grains) given to the cranes in order to reduce their damage to the fish raised in nearby ponds. Since the migration to Israel lasts about 3 weeks, it can be assumed that the cranes became infected in Israel or at a nearby destination on the way, probably from wild waterfowl that were clinically healthy but carry the virus.

To sum up the entire period since 2006 in which a highly pathogenic avian influenza H5-subtypes has been appearing in Israel every few years, it can be said that Israel is a portion of the global outbreaks especially those of Eurasia and Europe (46). The origin of these viruses is South East Asia and the transmission spillover is through migratory wild birds, mainly aquatic birds. Avian influenza outbreaks are integrated into the dynamics of the migratory seasons of the birds. Israel is located on migration routes and is therefore exposed to these viruses and to the phylogenetic evolution they undergo in the southward route from Europe to Africa and the northward route from Africa to Europe (13). It can be assumed that with the introduction of influenza viruses through wild birds during a new outbreak, the viruses spread in Israel reaches commercial poultry and zoos or animal parks raising ornamental birds through the migratory birds or the movement of resident birds. However, in some of the events,

spillover through people, poultry trading, vehicles and other mobile means may also have contributed to the spread of the virus between poultry premises in different settlements in Israel.

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