

1 **Avian influenza H5N1 in Egypt: What we know and what we have to know**

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20 Counts:

 Abstract: 163

 Text: 2342

25 **Abstract**

26 Egypt has experienced endemic outbreaks of avian influenza viruses (AIV) of subtype H5N1 in
27 poultry since 2008. Since then, these viruses continue to evolve. In 2006-2008, outbreaks were
28 reported mostly during the winter season, but in 2009-2012 they occurred year round. H5N1 AIV
29 succeeded in infecting not only domesticated birds but also many other avian species and mammals.
30 Infections of humans were accompanied by mutations in the hemagglutinin (HA) protein which
31 improved the binding affinity to human receptors but simultaneously retained its specificity for
32 avian-receptors. Vaccines were applied nationwide to control the disease in poultry. Meanwhile, the
33 viruses accumulated several point mutations in the HA immunogenic epitopes resulting in antigenic
34 drift and the establishment of infections in vaccinated poultry. The Egyptian H5N1 viruses remain
35 susceptible to oseltamivir, but genetic markers indicating resistance to amantadine have increased
36 dramatically. In this mini-review, we discuss recent findings on the H5N1 viruses endemic in
37 poultry and humans in Egypt, and suggest possible options to control the infection.

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51 **Key words:** Avian Influenza Virus, H5N1, Egypt, Evolution, Reservoirs, Antivirals, Vaccination

52 **Introduction**

53 In February 2006, avian influenza virus (AIV) of subtype H5N1, clade 2.2.1, was introduced into
54 Egypt presumably by wild birds ([Saad et al., 2007](#); [Aly et al., 2008](#)). Since then, these viruses have
55 caused devastating losses in the poultry industry and threaten public health ([Abdelwhab and Hafez,](#)
56 [2011](#)). Although data from surveillance and research on the Egyptian H5N1 viruses have been
57 accumulating in the last few years, there are still large gaps in knowledge regarding the evolution
58 and epidemiology of these viruses. In this short review, we focus on three aspects of H5N1 viruses
59 in Egypt, virus evolution, potential reservoirs and susceptibility to antivirals and disinfectants. We
60 also suggest options to combat the endemic situation of H5N1 viruses in Egypt.

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62 **Virus evolution**

63 Time is the influenza virus' quest to adapt to a new host, environment and climate. Since 2006,
64 H5N1 viruses in Egypt are circulating in unique niches that favour its entrenchment. The viruses
65 circulate in backyard holdings where avian and mammal species including humans are in intimate
66 contact ([Abdelwhab and Hafez, 2011](#)). Therefore, specific mutations may be acquired with high
67 probability to alter receptor-binding affinity and/or increase the replication efficiency of the virus in
68 a wide range of hosts, which has been recently confirmed in mice as a model for human infection
69 ([Watanabe et al., 2011](#)). Likewise, speculations were raised about the acquisition of species-specific
70 genetic signatures of the Egyptian H5N1 viruses in donkeys and ducks ([Abdel-Moneim et al., 2011](#);
71 [Ibrahim et al., 2011](#)). Also, genetic analyses indicated that the recent Egyptian H5N1 viruses
72 possess mutations that could lead to a sustainable transmission from human to human by airborne
73 droplets ([Neumann et al., 2012](#); [Perovic et al., 2013](#)) which is of great concern and must be
74 investigated in depth.

75 Circulation of these viruses in birds which had been vaccinated with H5 vaccines ranging from 78
76 to 98% HA protein homology to the field virus, as well as in non-vaccinated or inadequately
77 vaccinated birds resulted in different evolutionary pathways ([Abdelwhab et al., 2012](#); [Arafa et al.,](#)
78 [2012](#)). Viruses isolated from vaccinated poultry showed stepwise acquisition of mutations in the
79 immunogenic epitopes of the HA resulting in divergence at an increased evolution rate to escape
80 from the immunity induced by the employed vaccine strains. This immune escape resulted in severe
81 outbreaks despite continuing vaccination ([Cattoli et al., 2011b](#); [Arafa et al., 2012](#)). In contrast,
82 viruses isolated from non-vaccinated poultry had diverged at a lower evolution rate ([Cattoli et al.,](#)
83 [2011a](#); [Abdelwhab et al., 2012](#)) and developed a unique strategy to evade the innate immune

84 response and replicate at higher levels in infected cells (Ahmed, A. S. et al., unpublished).
85 Continuous update of the poultry vaccines to match circulating field viruses and establishment of
86 AIV H5N1-free zones or compartments could help to ease this complicated epidemiological
87 situation.

88 The adaptation of the H5N1 viruses to different environmental conditions in Egypt remains to be
89 investigated. In 2006-2008, seasonal oscillation of H5N1 in poultry was reported with high
90 prevalences in winter, very rare occurrence in early summer and absence in mid-summer ([Aly et al., 2008](#);
91 [Hafez et al., 2010](#); [Murray and Morse, 2011](#); [Arafa et al., 2012](#)). However, since 2009 the
92 prevalence of the virus was high year round particularly in backyard birds and live bird markets
93 (LBMs) ([Arafa et al., 2012](#); [El-Zoghby et al., 2013](#); [Hassan et al., 2013](#)).

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95 **Virus reservoirs**

96 **Household poultry.** In Egypt an estimated 250 million of poultry is kept in backyard holdings,
97 which amounts to about 23% of the total poultry production ([Abdelwhab and Hafez, 2011](#)) kept by
98 over 10% of the human population ([Meleigy, 2007](#)). Surveillance showed that, in contrast to
99 commercial poultry, the virus is endemic in backyard birds which play an important role in further
100 transmission of the virus to other birds in the commercial sector and in LBMs ([Aly et al., 2008](#);
101 [Hafez et al., 2010](#); [El-Zoghby et al., 2013](#)). Moreover, most of the human H5N1-infections had
102 been associated with contact to backyard poultry ([Kandeel et al., 2010](#)). To investigate the role of
103 this possible reservoir, a targeted surveillance of backyard holdings was recently conducted in 111
104 households during the mid-summer season in five provinces with the highest H5N1 incidence in
105 2010-2011. Only in one province, asymptomatic infections of native duck breeds were reported in
106 three different backyards while chickens and imported duck breeds were negative ([Hassan et al., 2013](#)).
107 This low incidence rate in backyard ducks may indicate a minimal contribution as a
108 reservoir. Therefore, until now it is not clear whether ducks are the “Trojan horse” of H5N1 AIV in
109 Egypt serving as endemicity reservoir or whether they are victims being infected from another
110 reservoir. Nevertheless, the rapid and random movement of live poultry in Egypt may result in
111 widespread dispersal despite a limited infection at source. Also, slaughtering or disposal of sick or
112 dead chickens and native ducks can influence the results of such surveillance. Thus, it is useful to
113 investigate susceptibility of native and foreign avian breeds to the Egyptian AIV H5N1.

114 From 2006 until 2009, a vaccination policy was established using commercially available vaccines
115 of the H5 serotype but not necessarily matching the circulating field virus. However, this
116 vaccination strategy was not successful in reduction of the prevalence of the virus due to low
117 vaccination coverage ([Peyre et al., 2009](#); [El Masry et al., 2013](#)). Moreover, vaccination can suppress
118 symptoms and, thus, mask the infection, while excretion of the virus from infected birds is not
119 prevented. These asymptomatic virus shedders pose a serious risk not only for maintenance of
120 infection in poultry, but also for human infections. Hence, vaccination of backyard birds in Egypt
121 had been stopped in 2009 ([El Masry et al., 2013](#)). However, alternative strategies to control the
122 disease in the backyard sector were not established. Therefore, there is an urgent need for
123 innovative approaches to control the infection and to better understand its dynamics in the backyard
124 sector.

125 **Live bird markets.** In Egypt, marketing of poultry depends mainly on trade of live birds.
126 Commercial poultry from several provinces is usually gathered in collection points and then
127 redistributed throughout the country. In contrast, backyard birds are usually sold in local or
128 traditional LBMs. In Egypt, around 16000 LBMs sell live or freshly slaughtered birds to consumers,
129 and over 4300 small slaughtering and de-feathering points sell freshly slaughtered or chilled birds
130 ([Hosny, 2009](#)). Surveillance has revealed that H5N1 AIV is endemic in LBMs year round
131 ([Abdelwhab et al., 2010](#); [El-Zoghby et al., 2013](#)). Consequently, LBMs are considered an important
132 reservoir of H5N1 viruses in Egypt. The majority of LBMs contain birds from the commercial
133 sector (mostly chickens) and from backyard holdings (mainly waterfowl). The H5N1 incidence rate
134 was particularly high in LBMs which sold ducks ([Abdelwhab et al., 2010](#); [El-Zoghby et al., 2013](#)).
135 The exact pathway of transmission of infections from/to LBMs is unknown, also because recording
136 of the source of birds and tracing back the infections are very difficult ([El Nagar and Ibrahim, 2007](#)).
137 The infection may be perpetuated in the LBMs or introduced with each new batch of poultry.
138 Although transport of poultry without prior laboratory testing to confirm an AIV H5N1-free status
139 is officially prohibited, illegal transport of birds is common practice. Although the efficiency of
140 closing LBMs to control AIV infections has been proven in Hong Kong and USA ([Bulaga et al., 2003](#);
141 [Kung et al., 2003](#); [Lau et al., 2007](#); [Yee et al., 2008](#); [Fournie et al., 2011](#)), total closure of
142 LBMs is difficult to achieve because the capacity of slaughterhouses is limited to a maximum of
143 30% of the total poultry production ([Abdelwhab and Hafez, 2011](#)). There are several potential
144 options to control the infection in LBMs. (1) To change consumer preferences by selling frozen
145 birds at lower prices can be supported by the government or the poultry industry. (2) To invest into
146 the establishment of slaughterhouses which may be encouraged through reduction of customs fees
147 or taxes. (3) To establish a one-day rest each week or month for cleaning and disinfection of LBMs.

148 (4) To trace the infection of poultry in LBMs. (5) To provide fair compensation of the owners for
149 the depopulation of infected birds.

150 **Humans.** As of December, 19, 2013 173 human infections with AIV H5N1 were recorded in Egypt
151 of which 63 were fatal ([WHO, 2013](#)). All infected humans but two had documented contact with
152 poultry and the vast majority of infections was due to direct contact (e.g. keeping, slaughtering)
153 with backyard birds ([Kandeel et al., 2010](#)). Nonetheless, the actual number of infected humans in
154 Egypt is probably higher than the laboratory confirmed cases. Asymptomatic infection of humans in
155 Egypt is of great concern ([Anon, 2009](#)) and there are suggestions that infection of humans with
156 viruses of lower virulence, which had evolved from highly pathogenic precursors, may have
157 occurred ([Fasina et al., 2010](#); [Perovic et al., 2013](#)). Recently, evidence for seroconversion of
158 apparently healthy people in Egypt was obtained ([El-Sayed et al., 2013](#)). Hence, the role of putative
159 asymptomatically infected humans may be worth elucidating.

160 **Other animate and inanimate reservoirs.** In addition to domesticated birds e.g. chickens, turkeys,
161 ducks, geese, pigeons, ostriches and quails, presence of Egyptian H5N1 viruses has also been
162 reported from common egrets, crews and herons, the most common feral birds in Egypt. Moreover,
163 the virus caused morbidity and mortality in zoo birds. In mammals, H5N1 viruses have been
164 isolated from humans and donkeys, and serological surveillance showed anti-H5N1 antibodies in
165 pigs, dogs and cats ([Abdelwhab and Hafez, 2011](#); [Eissa et al., 2012](#); [El-Sayed et al., 2013](#)).
166 Recently, AIV H5N1 genomes were detected by RT-PCR in freshwater fish. This contamination
167 was probably acquired through fertilization of fish ponds with infected poultry manure, feeding of
168 fish with infected poultry carcasses or presence of infected feral/backyard birds around the aquatic
169 habitats ([Eissa et al., 2012](#)). The potential spread of the H5N1 viruses into other birds and livestock
170 in Egypt was not investigated, so far. Likewise, there is little knowledge on the persistence of H5N1
171 viruses in the environment, particularly in poultry manure and water (e.g., ponds, canals, lakes and
172 seas), although it has already been considered a risk for increased H5N1 AIV infections of poultry
173 in Egypt ([Sheta et al., 2013](#)). The role of other animal species as reservoirs (e.g. rodents, wild birds)
174 or vectors (e.g. mosquitoes, houseflies) for H5N1 viruses in Egypt should be elucidated.

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176 **Susceptibility to antivirals and disinfectants**

177 In poultry, the incidence of the mutation in the M2 protein, S31N, resulting in amantadine-
178 resistance has dramatically increased since 2008 ([Kayali et al., 2011](#)). Almost all resistant strains
179 were isolated from commercial poultry farms in sector 3 which have minimal biosecurity measures,

180 if any. Whether those viruses are naturally selected or whether there is a positive selection by illegal
181 application of amantadine in poultry in Egypt is currently under investigation. It is worth
182 mentioning that amantadine is the cheapest anti-influenza drug and, thus, financially attractive also
183 for use in poultry. However, the extra-label use of amantadine in poultry is totally prohibited to
184 minimize emergence of amantadine-resistant strains in human ([Abdelwhab and Hafez, 2012](#)).

185 Luckily, the neuraminidase inhibitor oseltamivir is still effective in treatment of infections of
186 humans with Egyptian H5N1 viruses as only a few strains were shown to be resistant ([Earhart et al.,
187 2009](#); [Kandeel et al., 2010](#); [Kayali et al., 2011](#); [Younan et al., 2013](#)). Zanamivir is also effective
188 against the Egyptian H5N1 viruses ([Rashad et al., 2010b](#)). Many synthetic chemicals and herbal
189 extracts have been shown to inhibit replication of H5N1 AIV. Synthetic chemical compounds such
190 as thiophene, thienopyrimidine and pyrazolopyrimidine nucleosides, pyrazole, pyridazinone,
191 oxadiazole, triazole, thiazolidine and thioxopyrimidine derivatives had variable antiviral activities
192 against strains of H5N1 AIV in Egypt ([Rashad et al., 2010a](#); [Rashad et al., 2010b](#); [Flefel et al.,
193 2012](#)). Extracts from Capparis sinaica Veill, Aloe hijazensis and the Red Sea grass Thallasodendron
194 ciliatum have successfully inhibited replication of some Egyptian H5N1 viruses in vitro ([Abd-Alla
195 et al., 2012](#); [Ibrahim et al., 2013a](#); [Ibrahim et al., 2013b](#)). Also, fractions of esterified whey proteins
196 exhibited a potent antiviral activity against the Egyptian H5N1 viruses ([Taha et al., 2010](#)).
197 However, none have so far been examined rigorously under field conditions.

198 Moreover, commonly used chemical disinfectants e.g. Formalin, TH4, Virkon S and Glutaraldehyde
199 were effective to eliminate infectivity of the Egyptian H5N1 viruses even in the presence of organic
200 matter when used in the appropriate concentrations for two minutes at room temperature (Marzouk
201 et al., unpublished data). Nevertheless, the resistance of the Egyptian H5N1 viruses to
202 environmental conditions (e.g. temperature, humidity, salinity, dryness, PH) is still unknown.

203

204 **General remarks and recommendations**

205 In the foreseeable future the elimination of the H5N1 AIV infection endemic in poultry in Egypt is
206 unlikely. Therefore, more efforts are required to better understand changes in the evolution and
207 epidemiology of the virus. Several genetic analyses indicated that the Egyptian H5N1 viruses carry
208 several molecular markers that are linked to (1) adaptation to humans, (2) increased virulence in
209 mammals, and (3) human-to-human transmission. Thus, there is an urgent need to study the
210 virulence and transmissibility of these viruses in mammals e.g. ferrets, mice, guinea pigs as a model
211 for the human situation. Although oseltamivir currently remains effective against H5N1 AIV in

212 Egypt, constant monitoring for the possible emergence of resistant variants is required. Rigorous
213 evaluation of the potency of vaccines against Egyptian strains of H5N1 is essential to effectively
214 control the disease in poultry. Ideally, this should be conducted frequently either annually or
215 biennially under representative field conditions (e.g. efficacy against new emerging viruses and/or
216 in the presence of maternal immunity). Surveillance should continue in LBMs, backyard holdings
217 and humans to understand the ecological and biological reservoirs of the virus. Tracking the
218 infection in LBMs, establishment of new slaughterhouses and changing the consumer preferences
219 are a real challenge for the control of H5N1 AIV in Egypt which must be considered by the
220 policymakers. Control of the disease in the backyard sector should take into account the societal and
221 economical needs of the owners. Last but not least, continuing awareness programmes are helpful to
222 prevent acquisition and spread of the infection, particularly in the rural areas and suburbs.

223

224 **Acknowledgment**

225 This work was completed at the Institute of Molecular Biology, Friedrich-Loeffler-Institut, Federal
226 Research Institute for Animal Health, Germany in the frame of a postdoctoral fellowship granted to
227 E.M. Abdelwhab by the Alexander von Humboldt foundation.

228

229 **Conflict of Interest**

230 The authors declare no conflict of interests regarding the publication of this article.

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