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MAPPING INFLUENZA A (H5N1) VIRUS TRANSMISSION PATHWAYS AND CRITICAL CONTROL POINTS IN EGYPT

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Acronyms and abbreviations

AI	Avian influenza
CAHO	Community Animal Health Outreach
C&D	Cleaning and disinfection
CLEVB	Central Laboratory for Evaluation of Veterinary Biologics
CRP	Critical risk point
DOB	Day-old bird
ECTAD	Emergency Centre for Transboundary Animal Diseases
EGP	Egyptian pound (EGP 1 = USD 0.167 at the time of study)
EID_{50}	Median Egg Infective Dose
FAO	Food and Agriculture Organization of the United Nations
GMP	Good Manufacturing Practice
GOVS	General Organization for Veterinary Services
HH	Household
HPAI	High pathogenic avian influenza
LBM	Live bird market
LPAI	Low pathogenic avian influenza
MoALR	Ministry of Agriculture and Land Reclamation
NLQP	National Laboratory for Veterinary Quality Control on Poultry Production
ND	Newcastle Disease
RPWD	Risk Pathway Diagram
RTPCR	Real-time polymerase chain reaction
SAIDR	Strengthening Avian Influenza Detection and Response Project
SH	Slaughterhouse
SOPs	Standard operating procedures
SPF	Specific Pathogen Free
USAID	United States Agency for International Development

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The views expressed in this paper are solely of the authors and do not necessarily reflect that of FAO, USAID or any other official agency.

Executive summary

This report summarizes the results of a study undertaken in 2010 and 2011 to assess and map Influenza A (H5N1) virus transmission pathways in the poultry sector and critical control points along the poultry value chains in Egypt. In order to focus specifically on the factors that either increase the risk of spread of H5N1 HPAI disease or are critical in disease risk management, a risk pathways diagram is sketched to describe the pathways (transmission routes, carriers and mechanisms) for transmitting virus from an infected node throughout the poultry value chain to disease-free premises. To assess the risk, the probability of virus movement into and from each point along the risk pathway and the impact of disease transmission from an infected node[1] to the next node along the value chain were assessed separately. Estimates of probability and impact were based on revision of quantitative epidemiological data and descriptive information from various sources, such as FAO study reports, General Organization for Veterinary Services (GOVS) reports and scientific literature. To fully understand all factors that contribute to the risk of virus transmission and to gather real-time information on control measures, activities, and priorities and information about historical outbreaks of HPAI, meetings were held with different key stakeholders which included GOVS, veterinary directorates, district veterinary services, Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), National Laboratory for Veterinary Quality Control on Poultry Production (NLQP), poultry association and private veterinary practitioners, and visits made to sector 2 and 3 commercial farms,[2] small-scale household (HH) poultry farms, slaughterhouses, live bird markets (LBMs) and poultry shops, modern and traditional hatcheries, feed mills, veterinary pharmacies, poultry Borsa[3] and litter collection and composting points in eight high-risk governorates. The categories of risk involved were divided into very high, high, medium, low and very low. Factors and actions involved in increase or decrease of risk were included.

Analysis of the results revealed that for commercial farms, risk associated with the movement of people is considered highly significant due to weak farm gate control and decontamination activities. The very high risk (with low uncertainty) category includes vaccinators from outside the farm, day and part-time farm workers, and visiting veterinary practitioners. The high risk category includes drivers of feed delivery, and egg and litter collection vehicles, while the vehicles themselves represent medium risk with high uncertainty. The medium risk category includes medical representatives and drug suppliers. Equipment shared among farms, such as egg cartons, vaccine atomizers and bird crates represent high risk with medium uncertainty in the case of multi-age farms. Shared bird crates and gas cylinders represent medium risk with medium uncertainty for one-age farms. The

[1] Nodes are premises or fixed points in the risk pathway diagram.

[2] According to FAO's classification - Sector 1: Industrial and integrated poultry production system with high biosecurity; Sector 2: Commercial poultry production system with high biosecurity; Sector 3: Commercial poultry production system with low biosecurity; Sector 4: Village or backyard production with low biosecurity.

[3] In 2002, the Governor of Qalubeya, along with some dignitaries and poultry producers, decided to set up a so-called "Borsa" (similar to a stock exchange) to introduce a more "open and scientific" price setting system to break the exiting poultry brokers.

overall risk related to rodents, insects, dogs is very low , cats medium, but very high for wild birds. Feed and water inputs represent low risk.

In the small-scale household production, the risk associated with introducing newly purchased adult waterfowl and *Baladi* chicken without quarantine, the movement of non-resident commercial farm workers and wild birds with access to the feed and water of poultry flocks is very high. The overall risk associated with purchased young birds and exotic chicken is low and medium, respectively.

Litter collection points (litter is processed and used as fertilizer or fish feed in aquacultures) and feed mills represent very low risk nodes along the poultry value chain, and the former can be considered as an end point for the virus. However, both could act as disease pathways to and from different commercial farms because of the high frequency of movement of vehicle and drivers, and the poor application of cleaning and disinfection (C&D) measures by either commercial farms or stakeholders working at litter collection points or feed mills.

Slaughterhouses do not facilitate virus replication or shedding because they apply the "live-in, dead-out" policy and could eliminate the virus from the poultry value chain; as a result, they represent very low risk for the poultry value chain. The risk they could impose is contamination of the environment due to lack of drainage treatment system or absence of C&D for vehicles or crates.

Most sector 2 and 3 producers buy feed on credit until batch selling, and due to fear of loss they may not notify any cases of infection, to illegally sell infected birds and to hide and improperly dispose of dead birds. Some specialized traders actually profit from the disease by purchasing birds known to be infected at very low prices and reselling them via door-to-door peddlers or to the slaughterhouse, which in turn sells frozen birds to fast food outlets. Unsuspecting buyers, such as village women, and/or some fast food retailers with no or little risk awareness can facilitate this type of cheap trade and thus disease spread.

The absence of signs of overt clinical disease in some duck breeds has led some to argue that ducks are the "Trojan horses" of H5N1 in their surreptitious spread of the virus (Kim *et al.* 2009). In Egypt, many Trojan horses for H5N1 virus are in place: weak application of farm-gate biosecurity measures, unregulated wide use of variable vaccination protocols and programmes by commercial farms, co-circulation with H9N2, lack of awareness of small-scale household producers on the importance of quarantine of newly purchased birds and keeping birds in a confined environment, unregulated live bird trading, and weak movement control together facilitate "silent spreading" of H5N1 HPAI viruses, continuing the circulation and endemicity of the disease.

As long as birds are reared under management systems with poor biosecurity, including free movement without inspection or traceability, and an inefficient vaccination strategy, the spread and circulation of H5N1 HPAI will continue. Thus, critical control points for prevention of AI virus transmission along the poultry value chain include the quarantine of newly-purchased birds and keeping birds in a confined environment by small-scale household producers, strict farm-gate biosecurity by commercial producers, strict application of the "live in, slaughtered out" policy by LBMs, restructuring of LBMs in such a way as to permit sound decontamination and directional flow from dirty to clean zones, and efficient movement control by regulatory authorities.

Due to the high density of commercial poultry farms and small-scale household production in most governorates, there is a need for a national poultry production standards and guidelines to regulate and support good management. The system should enhance the application of biosecurity measures by the poultry production and trade sector and by the actors involved in the poultry value chain, with clear critical limits that must be met. Programmes based on a range of clear, scientifically justified principles suitable for the Egyptian situation and applicable to any level of poultry production, and auditable measures intended to prevent disease-causing agents from entering and/or leaving premises.

The formation or strengthening of grassroots producers' associations could be instrumental in improving the dialogue with the authorities on the development of incentives for the improvement of biosecurity and in facilitating monitoring, coordination, communication, transparency and agreement among poultry producers, even among competitors in the same region, and make the poultry sector work for all.

Introduction

Risk is defined as the possibility (likelihood) of an adverse event occurring and of the consequence of its occurrence (Murray *et al.* 2004; Zepeda 2007). Métras (2008) classified avian influenza as a modern risk or even a global terrorism that may change life significantly if considered uncontrollable and if it becomes omnipresent as a threat.

Following the emergence of Influenza A (H5N1) Highly Pathogenic Avian Influenza (HPAI) in Egypt in 2006, the authorities began culling birds and organized massive vaccination to control the spread of the disease in poultry. However, despite considerable attempts to control the disease, there have been a large number of outbreaks (2 494 declared cases) both in small-scale household and commercial poultry production units, as well as 167 human cases of Influenza A (H5N1) infection with 60 fatalities by April 2012. The current endemic situation in Egypt continues to constitute a major challenge to the country's poultry industry and regulatory authorities.

Understanding the epidemiology of the disease is a key element in HPAI control. It is essential to link epidemiological knowledge with action to effectively limit the spread of the virus. More effort should go into sharing and using epidemiological information, including socio-economic and geo-spatial data, for planning animal health programmes and decision making.

A critical risk point (CRP) is defined as a point, step, or procedure at which control can be applied and a disease hazard can be prevented, eliminated or reduced. CPR should be identified along production and marketing of the poultry value chains, where the risk of virus spread is higher. Development of control measures is essential to mitigate risk at these points. High-intensity of commercial and small-scale household poultry farming and high human density combined with weak hygienic and regulatory measures make Egypt a potential site for viral mutation and evolution. An understanding of the "usual" patterns of movements of animals, products, materials, people, vehicles, etc., is useful to know how disease could spread if introduced into different places along the poultry value chain. This in turn allows for strategic planning to reduce risks, to set priorities for resource allocation and its effective and efficient utilization, and to achieve higher benefit-cost ratios with existing or reduced resources (Thornton 2004; Stärk *et al.* 2006; FAO 2011).

Objectives

The objective of this study was to identify the risk pathways for the highly pathogenic avian influenza (HPAI) along the poultry value chain where disease transmission risks are higher, to determine the critical control points and the factors that increase the risk and to provide feedback to industry regulators in order to enable them establish intervention priorities at nodes with the highest risks as part of a strategic plan to monitor, regulate and control the disease in Egypt.

Methodology

Risk assessment, like all predictive models, is dependent upon both factual knowledge and assumptions. The greater the reliability on good data, the fewer assumptions have to be made, and subsequently the more reliable the assessment, if performed accurately. In few instances, where not all the facts known, it is necessary to make some assumptions when assessing risk. Risk assessment provides information for risk management and contains some or all of the following steps: hazard identification, dose-response assessment, exposure assessment and risk characterisation (Carver 2003).

In the present study, the focus was specifically on factors that either increase disease risk or those that are critical in disease risk management. The assessment included a review of the poultry value chain developed by animal production specialists, veterinarians and socio-economists for studies conducted in Egypt (Taylor 2007; Ibrahim *et al.* 2006; Ghonem 2007; Ibrahim 2007; Hosny 2008; Wilsmore 2008; Hosny 2009; Engelen 2011; Geerlings 2011).

Risk assessment first involves assessing the probability or likelihood of virus passing each step – identified in the poultry value chain – associated with different carriers. Probability estimation is based on release (pathways necessary to introduce [release] H5N1 virus into a disease-free premises) and exposure (pathways necessary for exposure of disease virus-free bird flocks to the disease) assessments (Murray *et al.* 2004). For probability assessment, quantitative epidemiological data and descriptive information from existing FAO study reports and documents, General Organization for Veterinary Services (GOVS) reports, current surveillance databases and published scientific literature were used.

Between May 2010 and April 2011, meetings were held with key stakeholders – GOVS, veterinary directorates, district veterinary services, Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), National Laboratory for Veterinary Quality Control on Poultry Production (NLQP), representatives of the Egyptian Poultry Association and private veterinary practitioners. Visits were made to commercial sector 2 and 3 and small-scale household poultry farms, slaughterhouses, LBMs and poultry shops, modern and traditional hatcheries, feed mills, veterinary pharmacies, poultry Borsa and litter collection and composting points in eight high-risk governorates ((Dakahlia, Gharbia, Monufia, Sharkia, Qalyubia, Giza, Fayoum and Minya) Fig. 1). The purpose was to formulate a comprehensive understanding of all factors that contribute to the risk of disease transmission and gather data HPAI prevalence and real-time information on control measures, activities and priorities. A risk pathways diagram was designed to describe and consistently assess the pathways (transmission routes, carriers and mechanisms) for transmitting the virus along the poultry value chain from an infected node into disease-free premises. In account of the prevailing information gaps, the qualitative analysis used by Defra (2002) and described by FAO (2011) was taken as a guide for estimating different levels of probability for virus transmission routes (Table 1). A combination of release and exposure risk categories using a matrix adapted from Métras (2008) was also used to allow categorisation of qualitative probability (Table 2).

Figure 1. Map of Egypt showing governorates (highlighted in pink) where HPAI risk is high and the study was conducted

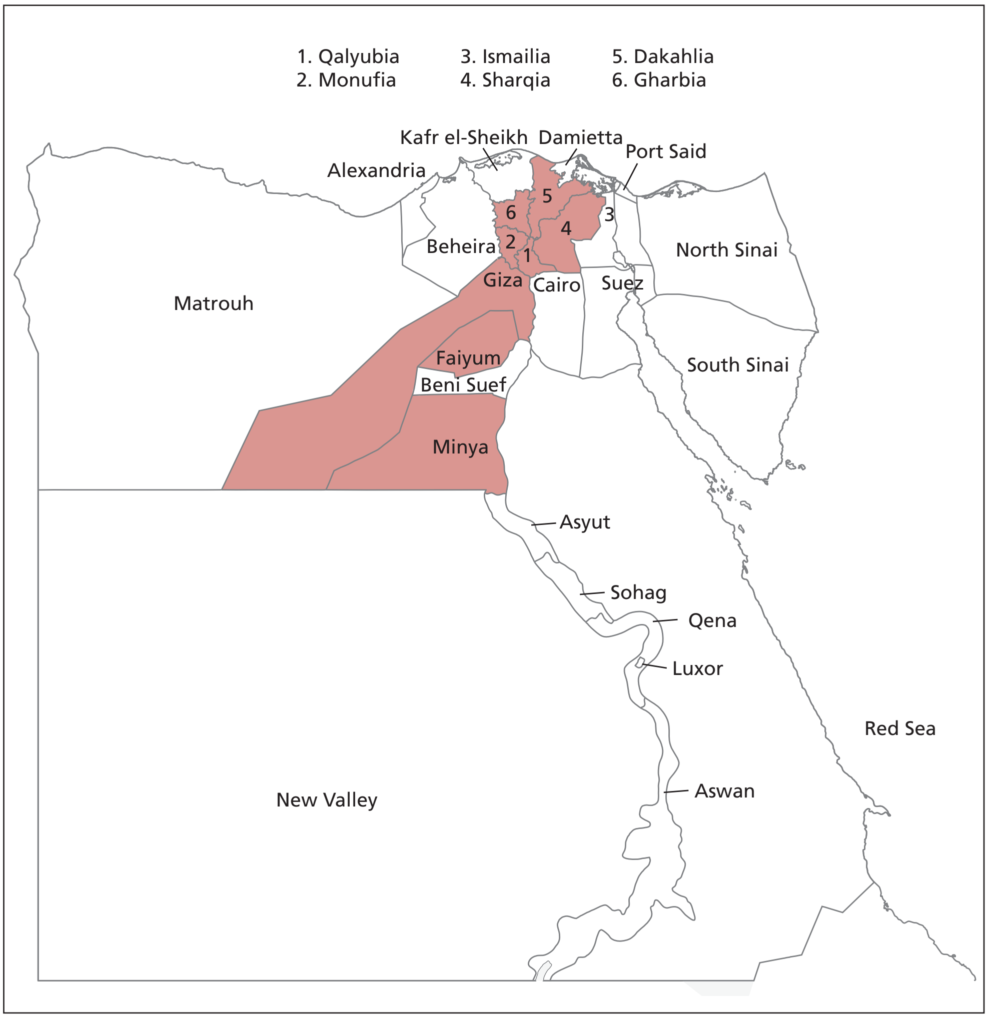


Table 1. Description of levels of risk probabilities

Probability		Description
VL	Very low	Rare (risky event may occur in exceptional circumstances)
L	Low	Possible (the risky event may occur in the next three years)
M	Medium	Likely (the risky event is likely to occur more than once in the next three years)
H	High	Almost certain (the risky event is likely to occur the current year or at frequent intervals)

Source: FAO 2011

Table 2. Qualitative measures of release and exposure likelihoods

		Likelihood of exposure			
		Very low	Low	Medium	High
Release likelihood	Very low	VL	VL	VL	VL
	Low	VL	VL	VL	L
	Medium	VL	VL	L	M
	High	VL	L	M	H

Source: FAO 2011

Table 3. Qualitative risk analysis matrix: risk levels

		Impact			
		Very low	Low	Medium	High
Probability	Very low	VL	VL	L	M
	Low	VL	L	M	M
	Medium	L	M	M	H
	High	M	M	H	VH

Source: FAO 2011

The consequence or impact of virus transmission from one node to another was estimated, and impact of risky events ranked on a scale similar to that used for probability (Table 1) from very low to high. The criteria used to assess impact were the potential for:

- amplification of infection (size of any resulting outbreak);
- spatial spread to new geographic areas;
- spread across species e.g., from duck to chicken or from poultry to human;
- losses (economic and livelihood losses) as a result of HPAI outbreak itself and of the control measures; and
- loss of human lives and welfare.

Due to the endemic situation of HPAI in Egypt[4], partly associated with high densities of human and poultry populations, the possibilities for virus amplification and mutation in adjacent flocks, loss of human lives and livelihoods, and economic losses are high. The impact of disease transmission from one node to another is therefore categorized at a high level.

Likelihood and impact using the matrices described by FAO (2011) and Defra (2002) were integrated to permit categorization of qualitative risk (Table 3).

According to the qualitative risk assessment scheme used by Defra in the United Kingdom, very high (VH) risks, had both a high probability of occurrence and high impact, demand immediate attention. Each risk category included the associated level of uncertainty (lack of precise knowledge on input data which could be due to data quality or lack of knowledge). Level of uncertainty was determined based on criteria summarized in Table 4.

Qualitative categories for expressing uncertainty in relation to qualitative risk estimates were adapted from Métras (2008). To avoid possible confusion, the steps in risk estimation are summarized in Fig. 2.

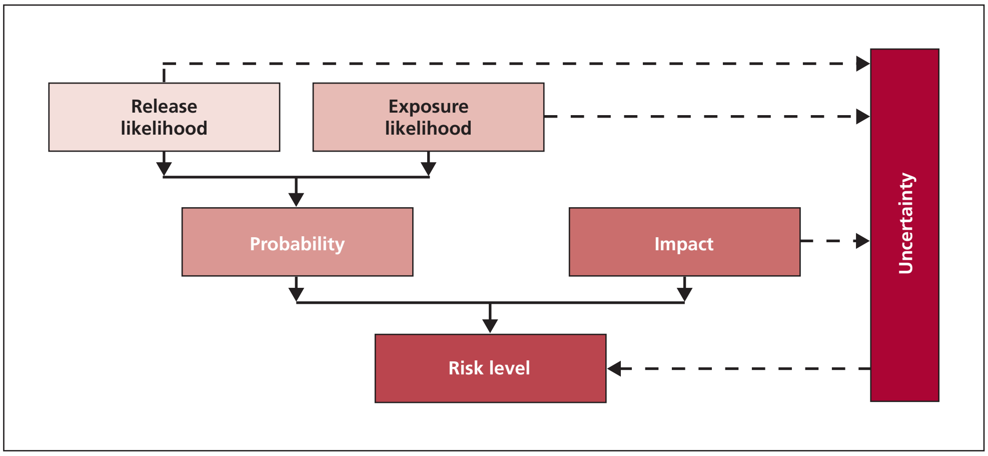
4 At the time of writing this report, 25 out of 27 governorates had been affected by HPAI.

Table 4. Qualitative measures of uncertainty levels

Uncertainty category		Descriptor
L	Low	Solid and complete data are available; strong evidence is provided in multiple references; authors report similar conclusions
M	Medium	Some but no complete data are available; evidence is provided in small number of references; authors report conclusions that vary from one to another Facts that can be seen/touched, e.g., the presence or absence of building, facility, etc.
H	High	Scarce or no data are available; evidence is not provided in references but rather in unpublished reports or based on personal communication; authors report conclusions that vary considerably from one to another

Source: Métras (2008)

Figure 2. Summary of risk estimation steps



Results and discussions

Poultry shed infectious A H5N1 virus into the environment in both nasal secretions and faeces. Previous studies on HPAI outbreaks have demonstrated that the most important mode of transmission in domestic poultry is related to the movement of humans, birds, contaminated materials and vehicles (Stegeman *et al.* 2004; McQuiston, *et al.* 2005; MoALR 2005; Thomas *et al.* 2005; Capua and Marangon 2006 and 2007; Bos *et al.* 2007; FAO 2007, Nishiguchi *et al.* 2007; Sharkey *et al.* 2008; Dorea *et al.* 2010; Spekreijse *et al*. 2011a). It has been demonstrated that the H5N1 virus survives from 4 to 23 days in wet chicken manure (Lu *et al.* 2003; Songserm *et al.* 2006), many months in cool water (Stallknecht et al. 1990; Zhang and Rogers 2006) and 72 hours on plastic, steel and rubber materials (Tiwari *et al.* 2006).

POULTRY VALUE CHAIN

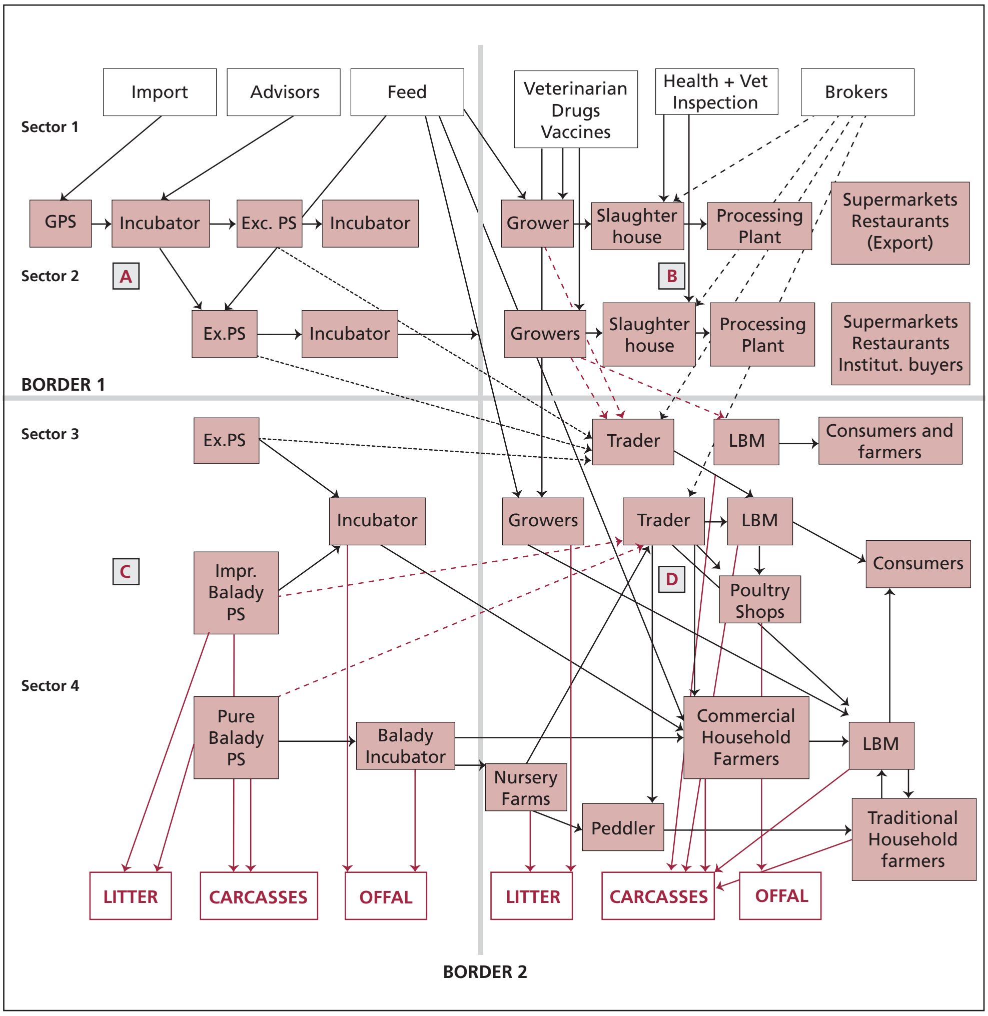
Engelen (2011) categorised the stakeholders engaged in the nodes of the poultry value chain in Egypt and identified 11 vertical stakeholders (poultry breeding companies, grandparent and parent stock companies, hatcheries, nurseries, layer and broiler grower farms, traders, slaughterhouses, processing plants, wholesale and retail outlets, and consumers), nine horizontal stakeholders (stock feed producers, veterinary services, vaccinators, brokers and Borsa, credit providers, extension, consultancy and training providers, transport, litter collection points, and branch organisations) and the interrelationships among them (see Fig. 3).

RISK PATHWAY DIAGRAM (RPWD)

The degree of connectedness of animal networks, which is the frequency with which links between different production premises and LBMs are made via people, animal movement and/or sharing of equipment, can determine the potential for widespread epidemics of disease (Kao *et al.* 2007). Fig. 4 shows the major nodes involved in the poultry industry, trading and transmission routes (pathways), and how they relate to each other and facilitate virus transmissions over the poultry value chain.

The diagram shows the possible pathways for virus transmission from and into a farm and/or household indicated by lines beginning with an assumed infected commercial farm/household and ending in arrows. The RPWD includes 10 fixed nodes, infected and clean farms, infected and clean houses, LBM/poultry shops, slaughterhouses and hatcheries, feed mills, litter collection points, fish farms and cultivated land. The movable carriers are people (part-time farm workers, vaccinators, de-beakers, feed suppliers, medicine suppliers, egg and bird collectors, poultry shop retailers, traders and visiting poultry consultants), live birds and animals, vehicles and equipment. To assess the risk, the probability of virus movement into and from each point along a risk pathway was assessed separately.

Figure 3. Poultry value chain in Egypt (Engelen 2011)



RISK ASSESSMENT

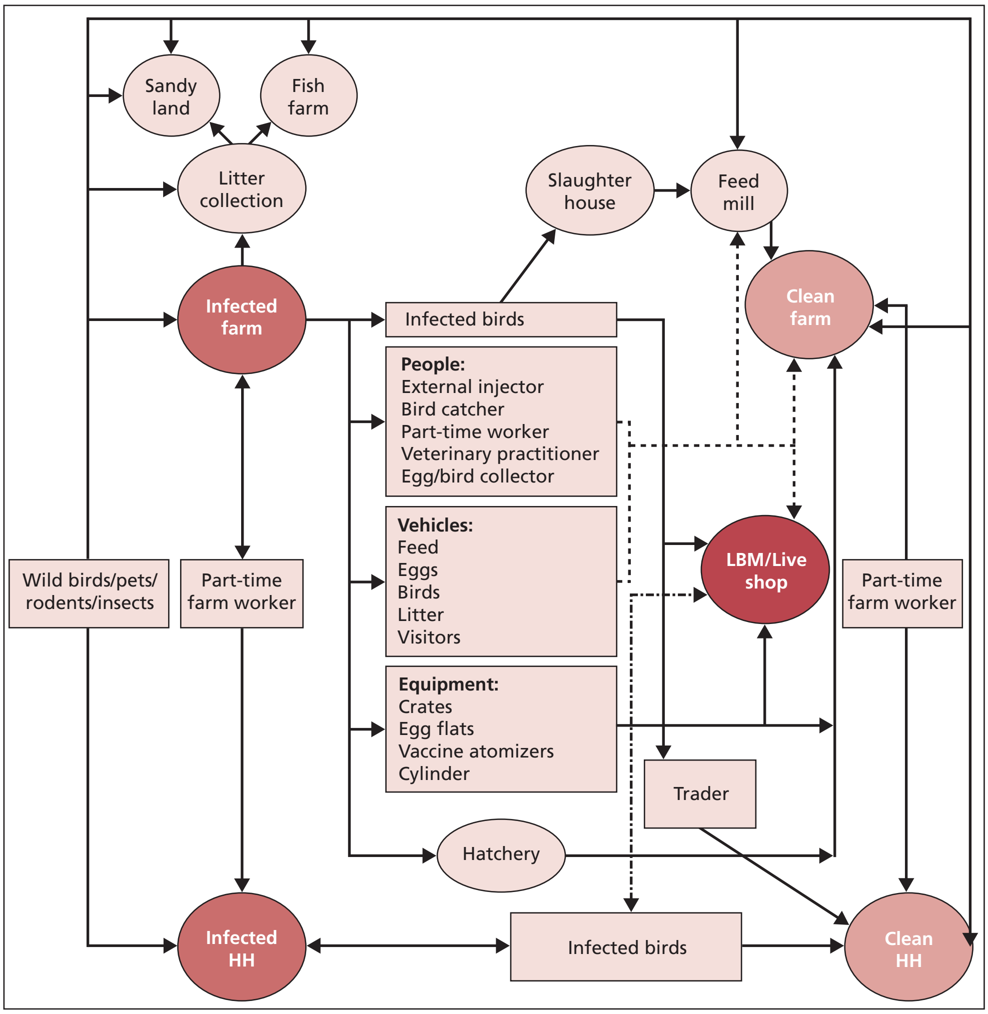
AI viruses are not highly airborne and their introduction in poultry farms mainly occurs through direct or indirect contact with infected birds, resulting from the movement of live poultry, people, vehicles, equipment or contaminated materials (Stegeman *et al.* 2004; Capua and Marangon 2006, 2007; FAO 2007; Spekreijes *et al.* 2011a).

Studies have shown that in the United States, Australia and the Netherlands, farms with most human movement, in particular farms that employed more outside workers or had vehicles come onto the farm and pick up dead birds, were more likely to be infected than others (Selleck 2003; McQuiston *et al.* 2005; Thomas *et al.* 2005).

Commercial farms

Egypt has a large poultry sector; and about 26 720 commercial farms (both registered and unregistered) that constitute the commercial poultry production industry and produce exotic broilers, indigenous (in Egypt referred to as *Baladi*) chickens, Peking, Muscovy, Mule and Sudani ducks, turkeys, ostriches, quails and eggs. About 81 percent of the broiler, 64 percent of the layer, 79 percent of the duck

Figure 4. HPAI Risk pathway diagram showing nodes, trading and transmission routes

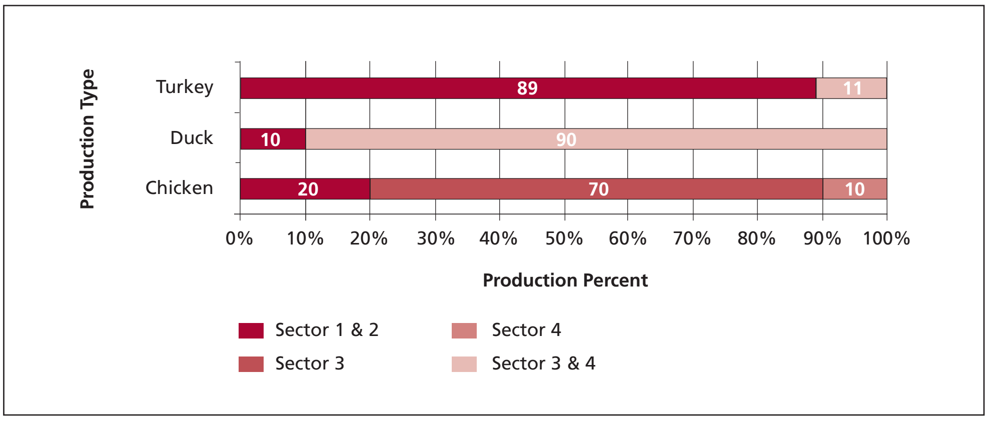


and 39 percent of the turkey farms are in Lower Egypt; representing the major share of the industry. Middle Egypt is the second largest with 13 percent of the broiler, 29 percent of the layer, 14 percent of the duck and 49 percent of the turkey farms. The rest are found in Upper Egypt (Jones 2008).

Commercial poultry farms provide about 90 percent of chicken produced in Egypt, with the remaining 10 percent provided by the small-scale householdpoultry farms that are abundant in villages and cities. About 74 percent of the broilers are produced on farms with less than 15 000 birds per cycle (Hosny 2006) (Fig. 5), while small-scale farms are the primary source for 70 percent of other poultry meats (principally ducks and turkeys). Total daily exotic broiler production varies between 1.6 and 2.0 million birds in the summer and winter seasons, respectively (AbdelGaid and Bakri 2009). All *Baladi* chicken and about 80 percent of the exotic broiler chicken are marketed alive via LBMs, poultry shops or door-to-door peddlers.

Egypt is considered an important “duck country”. There is an estimated 35–55 million duck population in the small-scale household sector and approximately

Figure 5. Contributions of the different production and farming systems to national poultry meat production in Egypt



5 million ducks in commercial farms (Hogerwerf and Siddig 2007). Around 280 000 day-old female Mule ducklings are imported from France on a weekly basis, nursed on commercial farms for 1–2 weeks, and then sold to small-scale household producers. On festive occasions such as Ramadan, commercial farms fatten ducks for about two months in time for markets. Annual turkey production in Egypt is estimated at 900 000 birds, of which 89 percent are imported mainly from France for fattening purposes.

People

Human movement among poultry farms was shown to be an important risk factor for avian influenza infection during the Virginia avian influenza outbreak in 2003 (Akey 2003; McQuiston *et al.* 2005). For the purpose of the assessment reported here, most actors who visit a farm have been categorised according to the risk level they contribute.

After an onset of signs of clinical illness in a flock, farm owners usually consult an external veterinarian seeking to know the cause of illness (diagnosis) and prescription of medicine. Widespread vaccination against H5N1 disease may mask the disease and render the clinical signs ambiguous (e.g., HPAI may be confused with fowl cholera or Newcastle Disease). In most sector 2 and 3 farms, this often leads to either injection of antibiotics or emergency Newcastle Disease vaccine spraying by external crews. The result is that veterinarians and external crews and their equipment/vehicles may carry the infection to clean farms. About 95 percent and 80 percent of short- (broilers) and long- (layers) cycle commercial farms, respectively assign external crews for vaccination and/or administration of antibiotics (FAO 2009b; Peyre 2011). A survey conducted by FAO (2009) on 147 commercial farms in Qalyubia governorate reported the use of 57 different external paraprofessionals, locally referred to as “injectors”. In the presence of a weak biosecurity system, such practices pose greatest risk to the poultry industry.

While people may act as short-term mechanical carriers (Bean *et al.* 1985; te Beest *et al.* 2011), external injectors represent a very high risk of disease transmission because of high levels of release and exposure and high impact with low uncertainty, based on high frequency of their movements on a daily basis between farms in different locations, species, ages and types of production, close contact with infected and non-infected birds, and the use of the same clothing and footwear on different farms (FAO 2009a). According to producers, many of the outbreaks of H5N1 HPAI that occurred in Egypt in 2006 were as the result of visits by external injectors.

Farms visited often give very minimal or no attention to personal hygienic measures. Ali *et al.* (2013a) found that 84 percent of the 304 farms studied did not provide clean clothes for workers or visitors and 71 percent had no showering facility. H5N1 HPAI viruses would normally be detected by higher than normal mortality during production or shipment of poultry, but not in vaccine-protected poultry which sometimes still shed the virus (Savill *et al.* 2006). The wide spread AI vaccination in commercial poultry sector in Egypt could promote enzootic transmission and spread of the disease by masking the presence of highly pathogenic H5N1 viruses. Suboptimal dosage may also facilitate evolution of increased virulence (Lee *et al.* 2004; Taha *et al.* 2008; Iwami *et al.* 2009). Thus, despite the fact that AI vaccinations being a useful tool in HPAI control, it brings additional risk enabling silent transmission, encouraging evolution of the virus and complicating disease recognition (Hinshaw *et al.* 1991). The risk raised by vaccinators will be higher in the Delta region due to the high density of poultry flocks in limited geographic areas. The same could hold true for paraprofessionals engaged in undertaking routine farm husbandry practices such as de-beaking.

In addition to daily part-time farm workers, bird catchers constitute a high risk for disease transmission between households and commercial farm production locations. Bird catchers are boys who live in nearby villages and are often assigned by farm owners to assist injectors/vaccinators and de-beakers. Sector 2 and 3 layer and breeder farms employ young women as part-time workers from neighboring villages to collect and rank eggs. The risk they contribute increases during the production period and depends on the number of collectors which is directly proportional to the production capacity.

The number of temporary workers involved in poultry production is about one million, representing 40 percent of the total labor force in the poultry industry (ElNagar and Ibrahim 2007; Freiji 2008). assuming that approximately one-quarter of those temporary workers are engaged directly in commercial production farms, then this figure divided by the total number of commercial poultry farms (26 720) and after excluding five percent of the farms that are at low risk (as estimated by Ali *et al.* 2013a), there are approximately 9.8 workers per farm who could potentially carry the A/H5N1 virus between commercial and small-scale household production sectors. This is particularly true in areas with high flock densities, such as Qaluybia, Sharkia and Giza governorates, where the mean flock density is more than 8 flocks/km^2 (Kaoud 2007). Thus, temporary workers represent a very high risk due to their movement between farms and close contact with vaccinated commercial and unvaccinated small-scale household bird flocks characterized by high release, exposure and impact, low uncertainty attributed to information from different producers owed their flock infection to those people.

Many private veterinarians and poultry consultants are assigned by sector 2 and 3 farm owners to visit their flocks on a regular basis for routine flock health inspection and early disease diagnosis. It is possible that visits to infected farms may not result in diagnosis of infection at the time of visit if clinical signs have been masked due to flock AI vaccination or birds still in the incubation period of the disease. Consequently, visiting veterinarians or consultants may fail to take the necessary precautionary measures before visiting other farms[5]. Thus, they are considered as very high risk due to their movements between different farms within short periods of time, and the level of uncertainty was categorised as low on the basis of information from flock owners. Farm visits by traders are considered to be one of the main risk factors for introduction of the H5N1 HPAI virus to poultry flocks. This means that the end of the poultry batch production cycle is a risk period for the introduction of diseases into a farm due to potential contact with contaminated crates or contamination associated with traders/drivers. The risk is further increased in large-scale or multi-age farms because it can take several days to sell an entire batch. Thus, if the virus is introduced into the flock during this period, it may spread silently within the batch and birds.

In Vietnam, Devaux *et al.* (2011) reported that the presence of at least one poultry trader in a village could be associated with the risk of HPAI transmission in the village. Further work in Hong Kong determined that the contact between the retail market and chicken farms via humans was a significant risk factor among chicken farms (Kung *et al.* 2007). Movement of egg collectors and truck drivers transporting feed and litter between different farms and locations increases the probability of release, exposure and impact to high, medium and high levels, respectively[6].

The risk associated with bird and egg collectors is high as they move between different farms, markets and locations and come into contact with potentially infected birds, or contaminated eggs, bird crates or egg flats. On the other hand, medical representatives and suppliers are considered a medium risk with high uncertainty because, although they move from farm to farm and other locations over long distances, they do not come into direct contact with infected flocks (Table 5).

<u>Vehicles</u>

Ali *et al.* (2009) showed that H5N1 HPAI virus had been transmitted over a long distance (over 700 kilometres) in a short time in Egypt. Balish *et al.* (2010) also showed that a new variant H5N1 virus had spread in a short period to at least seven governorates. Several types of vehicles that transport inputs or products are linked to different nodes of the poultry value chain. Service crew, egg and bird collecting and feed delivery vehicles are the ones that most frequently move among different farms. The frequency that a poultry consultant, an injector or an egg collecting vehicle visit a farm may vary from 1–6 days, but each one of them visits different farms each day. Feed trucks visit a farm every 5–30 days. In the case of litter collecting vehicles, the frequency of farm visits varies according to the farm type: 2–15 days (layer caged farms), 45 days (broiler farms), two months (*Shamourt*[7] farms), and 3–5 months (floor rearing breeder and layer farms). Similarly, the frequency of bird collecting vehicle visits to farms is variable depending on the length of the production cycle: 1.5 months for broiler, 2 months for *Shamourt*, and 20 months

5 For example, professional veterinarians involved in H7N7 outbreak control activities in the Netherlands in 2003 were found to play a potential role as vectors for disease transmission (te Beest et al. 2011).

6 According to layer and multi-age broiler farm producers, uncertainty was ranked as low, but high for litter collectors due to lack of information or reports.

7 *Shamourt* is a hybrid chicken, crossbred between exotic Saso and indigenous (*Baladi*), reared as a meat (brolier) type with a fattening period up to 60 days to reach a live weight 1.5-2kg/bird.

for layers and breeders. This frequency increases in the case of multi-age broiler or layer farms. Small and medium size vehicles are likely to be used in transporting fattened and spent birds, and for a patch of 5 000 broilers three large size or seven small-medium size vehicles are required, thus, one entire batch requires at least 2-3 days to sell. About 85 percent out of the 304 farms studied in 2010 had no wheel dip and 75 percent of those vehicles are parked close to the poultry houses (Ali *et al.* 2013a). It was found that the probability of transmission of infection between governorates increased because of active poultry transport but was not related to the distance between governorates (Kaoud 2007)[8]. Despite the high level impact of disease transmission among different farms and locations, the likelihood of release is high and exposure is low, and the outcome risk level according to the risk assessment matrix is medium with high uncertainty.

Equipment

Some equipment and materials (such as vaccine atomizers, gas cylinders, bird crates and carton egg flats) are frequently transported and shared among different production sites.Bird crates move with the highest frequency among different commercial farms, markets and locations. One crate is used to transport about 14 ± 2 exotic broilers or 19 *Shamourt* chicken. Therefore, for a cycle of 5 000 exotic broilers there is a need for about 357 crates. Plastic bird crates are rarely decontaminated by either traders or sector 2 and 3 farms.

Used egg flats with debris, feather, litter, bedding materials and faecal matter have been observed circulating among farms and between different locations. A study by Ali *et al.* (2009) revealed that about 63 percent of the 84 traditional hatcheries studied in Egypt receive fertile eggs in used egg crates provided by suppliers. Elsewhere, in California, some outbreaks of low pathogenic avian influenza (LPAI) were associated with transfer of virus by egg flats (Cardona 2005). The H5N1 HPAI virus has also been isolated from egg shells of vaccinated broiler breeders after challenge with a virulent virus (Abdelwhab *et al.* 2011). The H5N1 viral infectivity persists in the feathers of infected ducks for two weeks at 20 ^{o}C (Yamamoto *et al.* 2010), while H7N1 HPAI has been found in infected chicken subject to a post-mortem at 22-23 ^{o}C five to six days after death (Busquets *et al.* 2010). During the winter season, viability of the virus could remain for several days after release from the host (Sagripanti and Lytle 2007). Thus, used egg crates represent high risk because they are easily contaminated with the virus and may frequently move between farms and hatcheries. Egg setting in hatcheries is often made every three days, and in most instances eggs for different setting originate from various breeder farms in different locations.

Gas cylinders are used for heating poultry houses during winter and brooding of chicks. It is rated as having a medium risk level due to the exposure to direct sun light during transportation, and there is a low probability of a farm receiving a gas cylinder that had been used on another farm with medium uncertainty.

Bird crates constitute a low risk in commercial farms operating with mono-age birds, but high risk for farm with multi-age birds and LBMs with medium uncertainty.

[8] On an HPAI-infected farm in Thailand, Kasemsuwan (2009) reported that no virus was found in samples from wheels and trunk of vehicles, meaning that there was a low probability of vehicles being mechanical vectors.

Vaccine atomizers represent high risk because of contamination of their long electric cords by litter during use in emergency vaccination in chicken infected by Newcastle Disease, with high uncertainty.

Wild birds

It was suggested that that an HPAI virus may have been introduced into Egypt through a migratory bird (Normile 2006).This study is concerned with wild birds that have the ability to access poultry farms. Sparrows and doves are commonly observed wild birds that access poultry houses, feed stores, feed mills and litter collection points. Sparrows congregate in high density in and around poultry farms and are highly susceptible to the H5N1 HPAI virus. In addition, house sparrows excrete virus via the oropharynx and cloaca for several days prior to the onset of clinical signs (Kou *et al.* 2005; Boon *et al.* 2007; Brown *et al.* 2009; Poetranto *et al.* 2011). A study in China (Liua *et al.* 2010) highlighted the potential threat of this type of wild bird infection for veterinary and public health based upon isolation of the H5N1 HPAI virus from an apparently healthy sparrow. This isolate had close genetic relationship with the viruses that caused two human cases in the same province. In another study, house sparrows suffered only mild transient depression had no mortality and lacked gross lesions (Perkins and Swayne 2003b). H7N1 HPAI has been isolated from a collared dove in Italy (Capua *et al.* 2000) and H5N1 was isolated in 2004 in Thailand.

Researchers from the Ministry of Environment of Egypt and Naval Medical Research Unit 3 (undated) found a rock dove positive for H5 during surveillance in the period 2003–2008. Several species of zoo and feral birds in Egypt's Giza zoo were exposed to H5N1 AI virus infection in February 2006, and an egret and/or a crow have been suspected of transmitting the virus to the main zoo in Egypt (Abdelwahab and Hafez 2011). Waterborne transmission was found absent from experimentally infected sparrow in contact with chickens (Forrest *et al.* 2010).

Since data on the prevalence of the disease and sources of infection in these species are not available in this study, it was assumed that the possibility of sparrows and doves (which can access poultry houses) becoming infected from contaminated litter collection points or share drink or feed of infected small-scale household or commercial farms is medium and that the rate of intra-species transmission in these hosts is very low (Boon *et al.* 2007). Therefore ranked the risk represented by sparrows and doves ranked as very high due to high release and exposure, and the impact as medium uncertainty because data are not available on contact rate, frequency of contact of wild birds with domestic birds and prevalence of infection.

Rodents

Rodent species are found in most poultry farms in Egypt. Rodents are very prolific; they can have litters every three weeks with 8–12 offspring per litter. Breeding peaks in spring and autumn. Rats and mice are known carriers of at least 35 diseases, and constitute major carriers and reservoirs of poultry pathogens, including: influenza virus, infectious bursal disease virus, and *Pasteurella multocida, Salmonella typhimurium, Salmonella enteritidis* and other zoonotic bacterial, viral, rickettsial, parasitic and mycotic pathogens (Adams 2003).

Rats can cover 2–3 kilometres each night in search of food, but will not travel far if there is sufficient food available locally. Naturally occurring cases of H5N1 HPAI have not been reported in rodents (Cardona *et al.* 2009). In a single experimental case of inoculation with the H5N1 HPAI virus, rats developed no clinical disease or lesions and no virus was detected at any time after inoculation. In fact, they appeared to be entirely resistant to infection (Perkins and Swayne 2003a). However, it has been purportedly reported that H5N1-infected rats were found on an AI outbreak farm in Thailand in 2004 (Kasemsuwan *et al.* 2009). Rodents are ranked as presenting very low risk. This is because that they feed on rations and dead birds while exposure is already high and, release is very low. As a result, rodents have low impact with medium uncertainty because of their limited movement between farms.

Insects

House flies are commonly observed in poultry houses, feed stores, feed mills and litter collection points in the summer season. House flies have an average longevity of 34.2 days (Rockstein 1957). During the winter season they are in either the larval or pupal stage often under manure piles or other protected locations and warm summer generally provides the best conditions for their development. As many as 10–12 generations may develop in one summer, and the flight range of the house fly is between two and 20 miles. It is known that the common house fly (*Musca domestica*) can transmit more than 100 different pathogens such as viruses, bacteria, protozoa and developmental forms of parasite such as oocysts and eggs. Pathogenic organisms are picked by the mouth and other body parts of flies, and then transfer disease organisms from both inside and outside their bodies. Darkling beetles are commonly observed on poultry farms. One beetle can lay up to 800 eggs in a litter during a 42-day period (equivalent to the broiler production cycle). Eggs develop into larvae within four to seven days. The life cycle ranges from 42 to 97 days depending on temperature. The beetles accumulate in dark corners of manure or litter, especially under sacks, in bins or in places where feed is stored. Pupation occurs in the litter, soil and side walls of poultry houses. The beetles migrate frequently throughout the litter generally coming in contact with soil. Adult beetles and larvae act as reservoirs for many poultry pathogens and parasites, including avian influenza (Lyon, undated). Beetles have been observed feeding on carcasses of dead poultry and adult chickens, and chicks are more likely to eat the beetles (Lyon undated web-based commucation). Sievert *et al.* (2006) isolated H5N1 from house flies and Bean *et al.* (1985) isolated highly pathogenic H5N1 and H5N2 from house flies in a chicken house in Pennsylvania, USA. It has also been suggested that blow flies could be mechanical transmitters of H5N1 HPAI following isolation of the virus in a vicinity of an infected poultry farm in Japan in 2004 (Sawabe *et al.* 2006). However, the same team (Sawabe *et al.* 2011) later suggested that the viability of the influenza virus decreases steadily in the blow fly crop and intestine.

Mosquitoes increase in number from spring to late autumn seasons, especially near water ponds/canals and rice fields in Egypt. Although H5N1 HPAI virus has been isolated from mosquitoes trapped on an infected farm in Thailand, there are no reports that indicate mosquitoes transmit influenza virus either mechanically or after an extrinsic incubation period (Barbazan *et al.* 2008).

The role of insects in the transmission of HPAI is ranked as very low, primarily because their populations are generally highest in the summer when minimal disease outbreaks occur. In addition, movement of insects over long distances is less likely and the life span of insects is short. Their role is mechanical and there is no evidence of disease transmission from infected to non-infected farms by insects. A number of studies (Carver 2003; EFSA 2008; Hop and Saatkamp 2010) have reported that insects carry virus only over short distances, so the risk of release is very low, exposure risk is high and there is a very low impact with medium uncertainty.

Dogs and cats

It is common to see dogs and cats roaming around commercial poultry farms in sectors 2 and 3. Dogs are susceptible to H5N1 AI infection; they react with a transient rise in body temperature and in some instances with specific antibodies but infectious virus could not be re-isolated and transmission of virus through contact with dogs did not occur (Giese *et al.* 2008). However, Maas *et al.* (2007) concluded that dogs sub-clinically infected with H5N1 influenza may contribute to virus spread. Outdoor cats in areas affected by the H5N1 AI virus in wild birds are at risk of lethal infection (Klopfleisch *et al.* 2007). Under natural conditions, infection of cats with the H5N1 influenza virus may occur after contact with infected birds or their excrement without inducing clinical disease, Kuiken *et al.* 2006 experimentally showed that domestic cats can be infected with H5N1 through eating infected material and that these infected cats can transmit influenza to other cats. However, horizontal transmission between cats has not been observed (Leschnick *et al.* 2006).

The risk level of dogs is ranked as very low: dogs do not roam far from home and do not come into contact with flock birds, while the effect of dryness and solar radiation inactivates contaminated faecal materials; despite of cats usually bury their faecal, the risk level was ranked as medium, due to ability to transmit the virus. No cases of H5N1 infection in dogs or cats has been linked to transmission to other species (Yee *et al.* 2009). Pet animals carry virus only over short distance (EFSA 2008; Hop and Saatkamp 2010), so they are characterised by very low release, low exposure and very low impact with low uncertainty (Table 5).

Feed

Most sector 2 and 3 commercial poultry farms depend on feed mills for formulated ration supplies. The contamination of grains used in feed formulation may occur through droppings of wild birds. However, feed itself represents a low risk with high uncertainty for disease transmission because of the pressure and temperature conditions involved in feed production. In addition, the diluting effect on contaminated grains of other ingredients which is sufficient to inactivate or dilute viruses to a level that is insufficient for infection, leading to very low release risk, high exposure risk and medium impact.

Water

One of the riskiest practices in commercial poultry farms and small-scale household producers is throwing dead birds into river and water canals, contaminating water courses, underground water, wild birds and insects. Drinking water used in poultry farms is ranked as low risk, due mainly to the common practice of its treatment with chlorine (NegroCalduch 2010). The presence of micro-organisms in water also reduces the survival of the avian influenza virus (Zarkov 2006). Thus, there is a very low release level, high exposure and medium impact with low uncertainty.

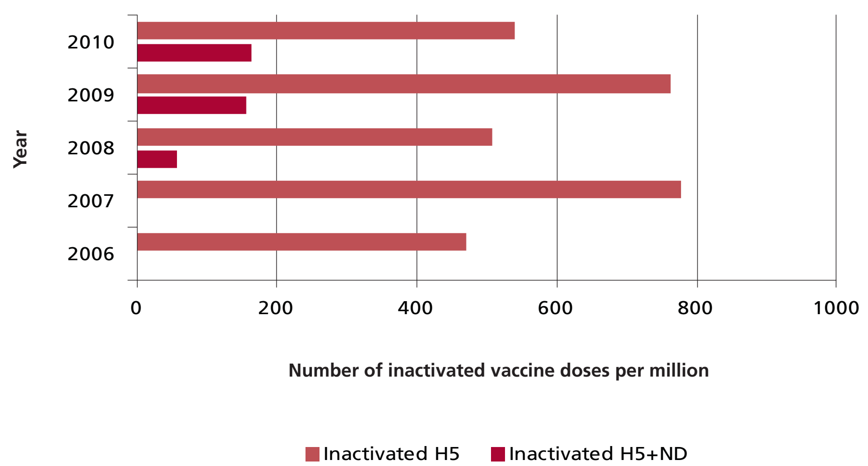
Biosecurity and vaccination practices and risk level

In Egypt, most sector 2 and 3 poultry farms are located near to or even in villages. In Qalyubia, Dakahlia and Sharkia governorates, where 63 percent of the commercial farms are very close to each other (less than 500 metres distance between farms), the transmission period for the H5N1 virus between districts was estimated at 2.3, 7.3 and 7.7 days in 2006, and this was attributed to the low levels of biosecurity (Kaoud 2007; FAO 2009a). Another study by Kaoud (2008) attributed 69 percent of epizootic cases that occurred in 2006 to biosecurity failure on the affected poultry farms. Pagani and Kilany (2007) identified poor biosecurity in sector 3 commercial farms and identified the high biosecurity risks to be inappropriate litter and carcass disposal, high density and close proximity of farms, inadequate quarantine of sick birds, contact with wild birds and contact of workers with other flocks. Thus, farms with low bio-containment measures represent threats to the industry chain. Based on farm biosecurity levels as shown by Ali *et al.* (2013a), *Baladi* chicken nurseries and *Shamourt* farms represent the greatest risk of disease spread in Egypt, compounded by the fact that birds are marketed alive for rearing purposes via door-to-door distributors or LBMs. Ali *et al.* (2013a) also reported that poor personal hygiene on commercial farms increase risk (approximately 84 percent of the 304 farms studied did not provide clean clothes for workers or visitors, and 71 percent of farms had no shower facility).

More than 0.7 billion doses of H5 inactivated vaccine have been used each year since emergence of the H5N1 virus in 2006 in Egypt (as shown in Fig. 6).

Shamourt farms mainly vaccinate against avian influenza without booster, and nursery farms do not vaccinate birds against H5N1. Partial immunisation due to

Figure 6. Number of H5 inactivated vaccine doses used in Egypt between 2006 and 2010



Source: Authors' personal communication with staff at CLEVB (Egypt) improper vaccination does not prevent HPAI infection or shedding and facilitates silent transmission and mutation of the virus (Domenech *et al.* 2009; Rudolfa *et al.* 2010). Similarly with exotic broiler farms, where some birds are sold to retailers who have no slaughtering and de-feathering facilities at village level, silent transmission may be facilitated due to partial immunisation by only one dose of vaccination. Avian influenza vaccine like all other inactivated vaccines requires 2–4 weeks following the first dose to achieve detectable and protective immunity in birds and during this period flocks are still susceptible to infection if exposed. When used properly, most influenza vaccines, including high quality products, prevent clinical signs but not infection (Swayne *et al.* 2001; Liu *et al.* 2003; Qiao *et al.* 2003; Savill *et al.* 2006; Capua and Alexander 2008). Poetri *et al.* (2011) concluded that single vaccination does not prevent H5N1 AI virus transmission among broiler chicken. The current vaccination programmes limit the probability of virus detection. Also, the wide spread of H9N2 LPAI in the country might complicate H5N1 virus recognition as the former could masks disease sings in HPAI infected birds (Khalenkov *et al.* 2009)

At the laboratory authorised to evaluate veterinary vaccines before release for market use, it was found that an inactivated H5 vaccine offered more than 80 percent protection for specific pathogen-free (SPF) chickens when challenged with a dose of 10^5 EID_{50}/ml, while the protection rate of the same vaccine fell to 30 percent and 10 percent, respectively when the challenge dose was increased to 10^6 EID_{50}/ml and 10^7 EID_{50}/ml (CLEVB, unpublished data). This indicates that the vaccine does not protect against higher levels of infection. Bean *et al.* (1985) reported that naturally-infected chicken may shed a high concentration of virus in their faeces (10^7 EID_{50}/ml per gram).

The high number of broiler flocks, increased number of birds and multi-age broilers per farm potentially increase the frequency of infectious contacts by traders, service crews, feed and DOC suppliers (Tsukamoto *et al.* 2007; Devaux *et al.* 2011). Suboptimal doses and absence of booster vaccination facilitate shedding of large amount of viruses therefore cannot block further transmission (Webster *et al.* 2006). It is important to note that even if a vaccine is used properly but coverage of vaccination is low, it is unlikely that outbreak of the disease will be stopped. The transmission efficiency of HPAI is quite high with a reproduction ratio of more than three (Van der Goot *et al.* 2005; Savill *et al.* 2006). Therefore, vaccine coverage in a given flock must be over 90 percent and all vaccinated birds must be protected to decrease the reproduction ratio to less than one. With this ratio, the transmission is unlikely to occur and thus outbreak is inhibited (Lekcharoensuk 2008; Bouma *et al.* 2009). However, Savill *et al.* (2006) demonstrated that even with 90 percent vaccination coverage using currently available inactivated vaccines might not prevent clinically "silent" field virus infections and further virus spread from such flocks. In addition, there are questions about the protective efficacy of the available vaccine in a field situation, and hence, the risks of silent spread of the HPAI virus. There is also concern that widespread and sustained but uncontrolled vaccination might facilitate antigenic drift and the selection of field virus variants which escape vaccine-induced immunity (Lee *et al.* 2004; Smith *et al.* 2006; Escorcia *et al.* 2008; Taha *et al.* 2008). In Egypt, about 25 percent and 80 percent of exotic broiler farms apply a one-dose vaccination protocol in summer and winter, respectively, and this is applied by less than 50 percent of *Baladi* raising farms (Peyre 2011).

Ali *et al.* (2013a) ranked poultry farms in Egypt according to biosecurity levels and reported that most of the farms studied (57 percent) were at high risk of infection, 38 percent at moderate risk and only about 5 percent at low risk. In terms of species and production type, turkey and breeder farms had the lowest risk, followed by layer, broiler, nursery and finally duck farms with the highest risk. In short, about 95 percent of farms are bio-insecure and vulnerable to exposure and release of infection. Capua and Marangon (2004) have highlighted how the use of vaccination alone without other stringent control measures pushes the situation toward endemicity instead of eradication, which reflects the current situation in Egypt. A long-term pattern of endemicity increases the opportunity for the emergence of potential pandemic strains through further adaptation by genetic mutation or re-assortment (Webster *et al.* 1992).

Due to weak biosecurity practices, one dose and variable vaccination programmes, lack of sentinel birds in flocks in commercial farms, absence of strict monitoring of unvaccinated sentinel birds by the authorities and live marketing of birds, nursery, *Shamourt* and exotic broiler farms represent a high risk for the poultry value chain, primarily for the small-scale household production sector, followed by the spent flocks of duck, layer and breeder farms. Infected broiler farms also represent a high risk for other commercial farms.

Despite the high risk due to the endemic situation, producers had a high level of awareness about HPAI, but limited knowledge on the value of vaccination, and variable knowledge on transmission and preventive measures. As a result, they ranked vaccination at the first priority for disease prevention. Therefore, prohibition of vaccine use by farms may be very difficult to establish due to the general belief among producers that vaccination is what is needed for disease control. Further, the marked decrease in mortality due to H5N1 HPAI among vaccinated birds may lead many vaccine importing agents to resist this policy and develop a black market. Given the fact that vaccination complicates disease recognition and facilitates silent transmission, and the difficulties involved in prohibiting vaccination, implementation and improvement of biosecurity is very important to achieve protection throughout the poultry value chain. Thus, biosecurity implementation should target governorates with high density of poultry farms. Risk-based surveillance, including the development of a protocol setting the priority of regular and pre-sale sampling for these farms is recommended.

> *Poor biosecurity application and vaccination in wide geographic areas are both considered Trojan horses for H5N1 virus because it masks the symptoms and birds shed virus while remaining asymptomatic; virus spreads freely with the movement of birds and persists along the poultry value chain.*

Table 5. Qualitative risk assessment for commercial poultry farms

Disease carrier		Likelihood of release	Likelihood of exposure	Probability level	Impact level	Risk level	Uncertainty	Notes
People	Vaccinators	High	High	High	High	Very High	Low	
People	Bird catcher boys	High	High	High	High	Very High	Low	
People	Egg collecting girls	High	High	High	High	Very High	Low	
People	Veterinary practitioner	High	High	High	High	Very High	Low	
People	Medical rep-resentative	Low	Very Low	Very Low	High	Medium	High	
People	Feed, egg and manure drivers	High	Medium	Medium	High	High	High	
People	Live broiler traders	High	Low	Low	Low	Low	High	In cases of one-age farms
People	Live broiler traders	High	Medium	Medium	High	High	Low	Multi-age farms
People	Spent live bird traders	High	Medium	Medium	High	High	Low	
Vehicle	Feed, eggs, manure	High	Low	Low	High	Medium	High	
Equipment	Gas cylinders	Medium	Medium	Low	High	Medium	Medium	
Equipment	Vaccine atomizers	Medium	High	Medium	High	High	High	
Equipment	Bird crates	High	Low	Very low	Medium	Low	Medium	One-age farms
Equipment	Bird crates	High	Medium	Medium	High	High	Medium	Multi-age farms
Equipment	Used egg racks	High	Medium	Medium	High	High	Medium	
Wild birds	House sparrows and doves	High	High	High	High	Very High	Medium	
Rodents		Very Low	High	Very Low	Low	Very Low	Medium	
Insects	House flies, mosquitoes and beetles	Low	High	Low	Very Low	Very Low	Low	
Dogs		Very Low	Low	Very Low	Very Low	Very Low	Medium	
Cats		High	Low	Low	High	Medium	Medium	
Feed		Very Low	High	Very Low	Medium	Low	High	
Water		Very Low	High	Very Low	Medium	Low	Low	

Small-scale household (HH) production

In 2007, out of the 17 300 000 total households in Egypt, 9 500 000 (55 percent) were rural dwellers (CAPMAS 2007). It is estimated that about 83 percent and 13.4 percent of rural and urban households, respectively, rear birds mainly for household consumption and as a source of income (Geerlings 2011). During 2008-09, the total poultry production was estimated at 250–300 million birds (Hosny 2006; Abdelwahab and Hafez 2011). About 71 percent of households in rural upper Egypt raise poultry; the average flock size in 2010 was 23.7 birds in contrast to 73 birds in lower Egypt (Geerlings 2011; Fasina *et al.* 2012). Chickens account for 48–52 percent of the total number of birds kept in the household sector. The ducks household population is estimated at 35–55 million, representing about 22–25 percent of poultry (Hogerwerf and Siddig 2007). Pigeons, geese and turkeys are other bird species raised in households.

The most important suppliers of breeding stock to the household sector are peddlers. The peddlers account for the supply of 71 percent of day-old chicks and 92 percent older birds (Geerlings 2011). Restocking often takes place during the months of March, April, September, October and the 2–3 months before Ramadan (Hogerwerf and Siddig 2007; Ali *et al.* 2013b; Geerlings 2011). *Baladi* chicken and exotic (Peking, Muscovy and Mule) ducklings are raised in nursery farms, then sold to household producers, while *Baladi* ducks, geese and pigeon are produced and raised at home.

Small-scale household poultry production usually operates throughout the year without any down time or an "all in, all out" practices. Birds are kept on rooftops or inside rooms in living houses. Flocks are made up of a limited number of birds of different ages and species, either scavenging around the house, or semi-ranging (i.e. allowed to roam for part of the day in yards or on rooftops) or strictly confined. Women are responsible for all husbandry activities including feeding, watering, bird care, marketing, etc. They provide feed and water twice a day (morning and afternoon). Drinkers are washed daily using water and palm fibres. Cleaning of manure/litter is performed every 2–7 days and manure is often collected in bags. Commercial feeds are given to young birds, but latter in the age birds depend on leftovers, crushed corn and for breeders old bread. In most instances, household flocks are composed of mixed species. Commonly chicken and ducks are reared together. Geese are separately raised because they fight with other species when mixed. On the other hand, pigeon are reared with all other poultry species as they occupy spaces near ceilings and pose no completion for space. Young birds are raised separately. Goats can also be found on rooftop *patios*.

Baladi chicks are purchased at the age of 3–4 or 6–8 weeks from door-to-door distributors, but one-day-old chicks are purchased directly from traditional hatcheries if the latter are located in villages. Peking ducks are purchased at the age of 2 weeks, while Mule or Muscovy ducks are purchased at one-day or 7-days old and fattened for household consumption and/or sold at LBMs to bird collectors. Eggs laid by *Baladi* ducks and geese are not consumed but used for in-house bird production. *Baladi* chicken is used for both meat (extra cocks and spent hens) and egg production. Sometimes, producers buy adult drakes or cocks from neighbours or LBMs for breeding or fattening for family consumption. They purchase commercial feed for young birds from retail shops on credit or cash, while they depend on home-produced corn/barseem/vegetables, and kitchen leftovers for adult birds. Some villages use topsoil (called rutch), and others rice straw or wood shavings as litter. The litter or manure generated is used as fertiliser. The meat and eggs produced are mainly used for home consumption. Sometimes eggs and/or live birds are sold to traders (who come one day before the village market date), to neighbours or directly to collectors in the LBMs.

Live Peking, nursed female Mule, spent, and *Baladi* drake and gander are commonly purchased in LBMs and adult *Baladi* chicken from peddlers for rearing, home consumption or as gifts to be donated on social occasions such as weddings. The tradition of duck-keeping in some village around the Nile Delta region is deeply embedded in the cultural life (Hogerwerf and Siddig 2007). Around 278 000 day-old female Mule ducks are imported from France each week.

In general, biosecurity is poor in this sector and inappropriate carcass disposal, high density of poultry farms, wild bird contact and worker contact with other flocks have been identified as high risks (Pagani and Kilany 2007). GOVS-CAHO reports and producer opinions attributed most outbreaks to newly purchased adult waterfowl and/or adult *Baladi* or hybrid chicken for rearing. Waterfowls have been widely recognised as a risk factor for disease occurrence, as a reservoir of infection (Gilbert *et al.* 2006; Pfeiffer *et al.* 2007; Fang *et al.* 2008; Biswas *et al.* 2009; Paul *et al.* 2010), and as a silent vectors for disease transmission (Chen *et al.* 2004; Hulse-Post *et al.* 2005; Sturm-Ramirez *et al.* 2005; Keawcharoen *et al.* 2008; Devaux *et al.* 2011). In Southeast Asia, HPAI H5N1 outbreaks were correlated with free-range duck farming and rice paddy cultivation (Songserm *et al.* 2004; Gilbert *et al.* 2006; Gilbert *et al.* 2008).

Vaccination of *Shamourt* or *Baladi* chicken by commercial farms, before being mixed with highly susceptible, unvaccinated house birds, masks signs of disease (Swayne *et al.* 2001; Tian *et al.* 2005; Domenech *et al.* 2009).

Birds infected with virulent avian influenza virus shed large quantities of virus in their faeces as well as in their saliva and nasal secretions. The incubation period for the disease before the onset of clinical signs could range from 2–10 days (Beato *et al.* 2007; van der Goot *et al.* 2008) and viral shedding can occur from 2–17 days after infection (Shortridge *et al.* 1998; Hulse-Post *et al.* 2005; Spickler *et al.* 2008). Chickens infected with the virulent strain shed high concentrations of virus in their faeces (10^7 EID_{50} per gram) (Bean *et al.* 1985). It has been shown that one infected duck excretes up to 10^{10} EID_{50} in 24 hours and, while ducks known to excrete 7.5–10 kg of faeces per year and geese to excrete 12.5–15 kg, infected waterfowl may be able to excrete up to 3×10^9 EID_{50} per gram in their faeces (WHO 2007). Spekreijse *et al.* (2011b) have reported that a low dose such as $10^{2.5}$ EID_{50} of H5N1 HPAI virus (clade 2.2) is infectious.

Vaccinated ducks infected with HPAI shed virus for longer periods of time, perpetuating the virus in the environment and increasing the possibility of transmission to susceptible birds (Wasilenko *et al.* 2011). Morbidity and mortality of HPAI H5N1 infection in ducks varies by age (Pantin-Jackwood *et al.* 2007) and is higher in younger ducks than in older birds (Kown *et al.* 2005). Between May and June 2011, five confirmed H5N1 cases in Egypt were isolated from ducks and geese from LBMs in Qalyubia (4) and Fayoum (1) governorates through an active surveillance conducted by GOVS (FAO AIDE News 2011).

Thus purchasing of waterfowl and adult *Baladi* or *Shamourt* chicken represents the highest level of risk due to high release, exposure and impact with low uncertainty. Trading of young birds (up to 3 weeks of age) represents a low risk with low uncertainty, because birds of this age are commonly reared separately from adult birds and the multiple vaccination of breeders against the avian influenza virus by commercial farms results in protection of progeny for the first three weeks of life (Table 6).

Wild birds such as sparrows or doves that share feed and water with unconfined flocks are ranked at a very high level of risk. Boys who work as bird catchers and part-time workers on commercial farms represent a very high level of risk with low uncertainty, while live bird collectors represent medium risk with high uncertainty. Shop/market slaughtered birds represent medium risk with high uncertainty for small-scale household production. Rearing and trading of pigeons represent very low risk levels because they are less susceptible to the H5N1 virus, show no clinical signs of disease or lesions associated with natural infection, and are less likely to transmit the virus to chicken (Lu *et al.* 2003; Perkins and Swayne 2003a; Liu *et al.* 2007; Werner *et al.* 2007; Brown *et al.* 2009).

Control activities should focus on raising awareness of small-scale HOUSEHOLD producers on the importance of quarantine of newly purchased birds, limiting contacts between birds and people, keeping birds indoor in a confined environment and proper bird disposal.

Table 6. Qualitative risk assessment of household production

Source of infection		Likelihood of release	Likelihood of exposure	Probability level	Impact level	Risk level	Uncertainty
Introducing domestic birds for rearing	Young birds: DOB (Mules, Muscovy ducklings, geese), Peking ducklings (up to 2 weeks old) and Baladi chicken (up to 3 weeks old)	Very low	Medium	Very Low	Medium	Low	Low
	Adult Baladi or Shamourt chicken	High	High	High	High	Very High	Low
	Adult Baladi duck or geese	High	High	High	High	Very High	Low
Exotic birds for consumption	Exotic broiler and Shamourt chicken	High	Very low	Very Low	High	Medium	Medium
Wild birds	Pigeons	Very low	High	Very low	Medium	Low	Low
	Doves and sparrows	High	High	High	High	Very High	Medium
People	Non-resident commercial farm workers and egg collectors	High	High	High	High	Very High	Medium
	Bird catchers	High	High	High	High	Very High	Medium
	Live bird collectors	Very low	Very low	Very Low	High	Medium	High

Live bird markets and poultry shops

Live bird markets are important sources for the purchase and trading of birds in rural and urban areas in Egypt and are prominent in the risk pathway diagram shown in Fig. 4. Poultry production is heavily dependent on LBMs and poultryshops due to consumer preferences and lack of slaughterhouse capacity (Hosny 2006; Ali *et al.* 2013b). LBMs and poultry shops absorb about 80 percent of total poultry commercial production in the country. About 36 percent of small-scale household producers sell their products (eggs and birds) to traders or neighbours in LBMs (Geerlings 2011). Due to cultural preference for consuming freshly slaughtered poultry, this type of marketing will not disappear easily (Ali *et al.* 2013b). There are many reasons why consumers prefer to buy poultry in LBMs, and include religious (slaughtering), social (feasts or welcoming traditions), and economic factors (absence of cold chain at home, higher price of dressed poultry, etc.). In addition, consumers simply wish to check the sanitary status of the bird and the freshness of the meat by themselves. In a global context, where poultry traceability and sanitary regulations are not considered sufficiently secured, it is rational for consumers to access the shortest distribution circuits to have assurances of quality (Fermet-Quinet *et al.* 2007).

There is a large LBM in every district and smaller LBMs for groups of villages. LBMs are usually weekly markets which take place in narrow and crowded streets in villages. Although their exact number is not known, they could be several thousands. Taking into account one per village, the estimate could be 5 000 (Fermet-Quinet *et al.* 2007). Officially, there are around 16 000 retail poultry shops registered in Egypt, in addition to the 4 300 small slaughtering and de-feathering points which are usually annexed to the back of the retail poultry shops (MoALR 2005). Unofficially, the overall number could be around 45 000 (ElNagar and Ibrahim 2007; Fermet-Quinet *et al.* 2007). Most LBMs continue to operate illegally in all governorates studied (Hosny 2008) with weak infrastructure, no C& D facilities, poor supervision and poor bird inspection, and often with sick and stunted birds. They sell eggs and live birds – young and adult chickens, ducks, geese, turkeys, pigeons, quail – for consumption and rearing purposes. Traders move with their birds between different LBMs which are held on different days of the week, buying and selling birds.

Live birds are presumed to constitute the highest risk because of virus replication, virus shed into the environment and movement over long distances in different directions (Cardona *et al.* 2009). HPAI surveillance programmes in several countries in Asia, including China, Hong Kong, Indonesia, Thailand and Viet Nam have demonstrated that HPAI H5N1 circulates in LBMs (Guan *et al.* 2002; Senne *et al.* 2003; Nguyen *et al.* 2005; Kung *et al.* 2007; Amonsin *et al.* 2008; Indriani *et al.* 2010). In north-eastern United States, LBMs have been found to be potential reservoirs for long-term maintenance of avian influenza viruses (Senne *et al.* 2003). In China, 16 viruses of different virulence and genotypes were isolated in late 2006 and early 2007 (Chen *et al.* 2009). Wang *et al.* (2006) and Indriani *et al.* (2010) isolated viruses from bird cages and Wang *et al.* (2006) detected neutralising antibodies against H5N1 in one out of 110 persons in the poultry business markets.

Live markets, which are considered to be a continuing source of influenza because of the dense concentration and high turn-over rate of live birds, provide ample conditions for virus amplification and may therefore be important reservoirs for HPAI or "hubs" of circulation (Webster 2004). Previous studies have shown that regular cleaning with detergents, including free chlorine concentrations typically used in drinking water treatment, can rapidly remove avian influenza virus contamination from surfaces (Rice *et al.* 2007; Trock *et al.* 2008), while periodic market rest days coupled with thorough cleaning can minimise the reservoir of virus in LBMs (Bulaga *et al.* 2003; Kung *et al.* 2003; Mullaney 2003; Guan *et al.* 2007).

Daily removal of waste and zoning that segregates poultry-related work flow areas have been found to be factors of protection (Indriani *et al.* 2010). A study in Indonesia showed that poultry water, drains, table tops, cages, tablecloths, utensils, bins and floors of LBMs were all contaminated. Environmental sites most commonly contaminated were located in slaughter zones and zones where carcasses were taken after slaughter, such as sale and waste disposal zones. Such contamination can be expected because slaughtering generates droplets that may contain viral particles and exposes internal organs with potentially high viral loads. Even if slaughtering is conducted in a separate zone, contamination can spread to the sale and waste disposal zone through carcasses and through the process of evisceration usually conducted in both slaughter and sale stalls (Indriani *et al.* 2010).

In Egypt in 2008, FAO (2008) found that four markets out of ten in Tanta district, Gharbia governorate, were positive to the H5N1 virus. By January–April 2009, 71 out of 573 LBMs examined (12.4 percent) were found positive for the H5N1 virus. A higher incidence (40.8 percent) of positive LBMs was recorded during the cold month of February and was concentrated mainly in the highly populated Nile Delta (Abdelwhab *et al.* 2010). Another study in the same year in the period January–September (Hany 2009) found 84 out of 197 LBMs (43 percent) tested positive for H5N1. Positive markets were scattered over 18 out of a total of 21 governorates tested. Between May and June 2011, five confirmed positive cases were detected in Qalyubia (four cases) and Fayoum (one case) governorates through the active surveillance programme conducted in both governorates by GOVS. All five were isolated from ducks and geese. There is no information or study on genetic analysis of these isolates or on the genetic relationship of these isolates with isolates from commercial and small-scale household birds.

The live bird market is one of most critical points in the poultry value chain. It links commercial and small-scale household producers, traders and consumers; the estimated volume of daily live exotic chicken traded ranges from 1.3 to 1.6 million birds in the summer and winter seasons, respectively, with maximum trading during Ramadan month (Ibrahim *et al.* 2006; Ali *et al.* 2013b). Lack of notification, bird tracing systems, registration of intermediaries, traders, peddlers transporters and retailers, in addition to minimal, if any, veterinary inspection in traditional LBMs facilitates movements of diseased and low quality birds from commercial farms to small-scale household production units and among small-scale household producers.

Permanent poultry shops in LBMs and poultry shops represent a low level of risk in the poultry value chain because most birds are "live in, dead out", species are caged separately, birds are kept for a short time before slaughter (1–2 days) and unsold birds remain in the shop (Ali *et al.* 2013b). Slaughtered birds are allowed to bleed, then submerged in hot water for scalding and mechanical de-feathering; the minimum temperature for inactivating the avian influenza virus in poultry meat is one minute at 80 °C, five minutes at 70 °C and 30 minutes at 60 °C (Moses *et al.* 1948; AQIS 1991), but improper handling of waste and drainage still contaminate the environment. Small slaughtering and de-feathering points represent medium risk with high uncertainty based on movement of these points between markets and unsold birds returned home.

With trading of ducklings aged 8–15 days, geese and *Baladi* chicken up to 3 weeks of age, the risk is low with low uncertainty due to the massive vaccination of breeder flocks which results in high maternal immunity in the progeny (only one outbreak reported in imported Mule ducklings[9]). These age groups are commonly reared in houses separately from the rest of the flock (Fasina *et al.* 2012).

Ducks constitute the foremost host of the avian influenza virus in general, and may shed virus for up to 2.5 weeks, while hardly showing signs of disease, hence contributing to persistence of the virus, at least in the Asian setting. Egypt has 35–55 million and five million ducks in small-scale household and commercial farms, respectively (Hogerwerf and Siddig 2007) and most ducks are marketed alive. Live adult Peking, and spent duck drake and gander are commonly traded in LBMs and adult *Baladi* chicken that are bought from peddlers for small-scale household rearing, consumption or donation as gifts on social occasions such as weddings represent a very high level of risk (with low uncertainty) for sector 4 (Table 7). CAHO reports and GOVS have attributed most small-scale household outbreaks to newly-purchased adult waterfowl and adult *Baladi* or hybrid chicken for rearing.

Waterfowl have been recognised as a risk factor for disease occurrence (Gilbert *et al.* 2006; Pfeiffer *et al.* 2007; Fang *et al.* 2008; Biswas *et al.* 2009; Paul *et al.* 2010) and as a reservoir of infection (Hogerwerf and Siddig 2007). Wasilenko *et al.* (2011) reported that vaccinated ducks infected with HPAI shed viruses for longer periods of time, perpetuating the virus in the environment and increasing the possibility of transmission to susceptible birds. Devaux *et al.* (2011) identified ducks as silent carriers.

According to Kung *et al.* (2007) contact between the retail market and chicken farms via humans is a significant risk factor among chicken farms in Hong Kong. In Egypt, it is likely that birds and traders move between different markets without inspection before market entry, unsold birds are returned home, people buy birds without being aware that they are infected and mix them with home flocks without spatial or temporal quarantine. To assist in preventing the spread of HPAI in Hong Kong SAR, a complete segregation policy was imposed in 1998 for domestic fowl (Sims *et al.* 2003). Currently, it is still illegal to rear, transport or market ducks and geese together with other poultry.

LBMs require strict application of a "live in and slaughtered out" policy, separation among different species, and separation between young birds for rearing and adult birds for consumption in areas suitably structured for application of sanitary measures.

[9] CAHO reports, GOVS.

Table 7. Qualitative risk assessment of LBM

Risk presenter		Likelihood of release	Likelihood of exposure	Probability level	Impact level	Risk level	Uncer-tainty
Permanent poultry shops and poultry shops in LBM		Low	Low	Very Low	Medium	Low	High
Small slaughtering and de-feathering points		Low	Low	Very Low	High	Medium	High
Live bird trading	Young birds duckling, geese and chickens aged up to 3 weeks	Very low	Low	Very Low	Medium	Low	Low
	Adult duck and geese	High	High	High	High	Very High	Low
	Adult Baladi	High	High	High	High	Very High	Low
	Adult Shamourt	High	High	High	High	Very High	Low

Litter collection points

Poultry litter is a combination of poultry manure and bedding material (wheat straw or wood shavings). The nutrient concentration of the litter depends on the type and amount of bedding material, production type (short [broiler] or long cycle farm), and the nutrients included in the poultry diet. Litter is purchased by litter collectors, transported on open-back trucks, covered by waterproof covers and collected in an open area. Litter is not normally treated before dispatch at either the farm or the collection point. Poultry litter is a good source of nutrients and organic matter for crops (Marsh *et al.* 2009) and in Egypt is widely used on sandy desert soil to improve its structure and consequently increase soil water-holding capacity. Rappaport and Sarig (1978) reported that the addition of chicken manure to an intensive fish culture results in reduction of the food conversion rate and Djajadiredja *et al.* (1980) reported that poultry manure is known to be the most powerful fertiliser for fish ponds. In Egypt, breeder and layer litter are preferred for fish farms because they are considered nutrient-rich due to the quantity of spilled feed they contain.

Movement of vehicles among different farms and locations without C&D performed at farm and collection point levels clearly present opportunities for the dissemination of avian influenza viruses to poultry, fish farms and crop land (Engelen 2011). The trading of unprocessed manure has been identified as potentially high risk (Mcleod *et al.* 2009). Kandun *et al.* (2010) reported a human case of H5N1 infection in Indonesia where exposure to H5N1-infected animals could not be established, but further investigation found that chicken faeces contaminated with viable H5N1 virus in garden fertiliser as the source of infection. Terregino *et al.* (2009) found that some H7 subtype strains are more resistant than others and remain viable after 15 days at 37 °C. However, Shortridge *et al.* (1998) reported that while the H5N1 virus can survive in wet faeces for weeks, it is inactivated as soon as the faeces dry out in ambient temperatures. As such, the spread of avian influenza viruses like H5N1 is expected to be relatively low in outdoor and free-range settings.

Risk levels could be high for caged layer farms resulting from the relatively higher frequency of litter vehicles and drivers visiting farms (every 3–7 days on average). Risk levels are also high for long cycle farms because most are multi-age production farms (litter collection every ≥3 months), and low for broiler farms (Table 4). There is no firm information that avian influenza has been disseminated in fish farms, however this possibility should be considered (Feare 2006). The risk for fish farms is considered very low, as it is also for cultivated lands because of the drying effect of direct sunlight.

Uncovered litter in an open area attracts wild birds. Egrets, sparrows, doves, hoopoes and pigeons are common wild birds observed in litter points. Sparrows, doves and egrets are susceptible to H5N1, but the latter never access poultry houses. In nature, hoopoes and pigeons have not shown H5N1 isolates. Thus, risk for wild birds ranges from very low for hoopoes, low for pigeons and medium for egrets, to very high for sparrows and doves, respectively (Table 8). The uncertainty for these risk estimates ranges from medium to high because of lack of data on number and capacity of such collection points, average storage time for litter batches, the seasonality of maximum activity of these points, H5N1 virus prevalence or its viability in these collection sites, or the status of the H5N1 virus in wild birds which feed on the litter at these collection points. It has, however, been shown that higher volumes and increased frequency of movements occur during the month of Ramadan (Ibrahim *et al.* 2006)

So litter collection points could be disease pathway in-between different commercial farms due to higher frequency of movement of vehicles and drivers through poor C&D application by commercial farms and litter collection points and disease pathway for small-scale household production and commercial farms through wild birds.

Table 8. Qualitative risk assessment of litter collection points

Points at risk		Likelihood of release	Likelihood of exposure	Probability level	Impact level	Risk level	Uncertainty
Commercial farm	Short cycle with "all in, all out" system	Low	Very low	Very Low	High	Low	High
	Multi-age broiler farm	High	Medium	Medium	High	High	High
	Long cycle farm	High	Medium	Medium	High	High	High
	Caged layer	High	Medium	Medium	High	High	High
Fish farm		Very low	Very low	Very low	Low	Very low	High
Cultivated land		Very low	Very low	Very low	Low	Very low	Medium
Wild birds	Sparrows and doves	High	High	High	High	Very high	Medium
	Egrets	High	Very low	Very low	Low	Very low	Medium
	Hoopoes	Very low	Very low	Very low	Very low	Very low	High
	Pigeons	Very low	High	Very low	Medium	Low	Low

Hatcheries

There are three types of hatcheries in Egypt, and are composed of 198 modern, 942 traditional and 10 semi-automatic (MoALR 2011). Traditional hatcheries and semi-automatic hatcheries incubate either *Baladi* chicken, Peking and Muscovy eggs. Modern hatcheries incubate either exotic, *Shamourt* chicken eggs or duck eggs (Ali *et al.* 2009). Egg setting occurs every three days, especially in winter when there is a higher demand for day-old birds (DOBs), which requires purchasing egg batches every three days from different breeder farms. The biosecurity measures applied by hatcheries are generally weak, except for those in sector 1. The main risk related to hatcheries concerns circulation of used egg racks and egg trucks among different breeders, species, farms and locations. This was a routine practice for 63 percent of traditional hatcheries in 2008 (Ali *et al.* 2009). Used racks may be contaminated with feathers and/or faecal material from infected farms. Yamamoto *et al.* (2010) found that viral infectivity persists in the feathers of infected birds for two weeks at 20 °C, and in California some outbreaks of low pathogenic avian influenza have been associated with transfer of virus by egg flats (Cardona 2005). HPAI H5N1 virus has been isolated from the egg shell of infected broiler breeders (Abdelwhab *et al.* 2011). Thus, hatcheries can play a role in virus transmission among different farms in different locations through contaminated egg flats, vehicles and drivers.

> *The critical practices are circulation of egg racks and the lack of decontamination facility for drivers and vehicles at farm or hatchery gates.*

Slaughterhouses

There are three types of slaughterhouses in Egypt and are composed of 37 fully automatic (14.4 percent), 37 semi-automatic (14.4 percent) and 183 manual (71.2 percent) (MoALR 2011). At the start of the HPAI outbreak in 2006, there were 184 slaughterhouses in the country. The 257 slaughterhouses today have a total annual potential slaughtering capacity of 187 million broilers, representing around 35 percent of exotic broilers and less than 20 percent of the potential annual broiler production (MoALR 2005; Ali *et al.* 2013b), with no adaptations to process spent layers, broiler breeders or native birds (Hosny 2006). Only 20 percent of broiler production reaches the consumer either frozen or chilled; less than 5 percent is further processed and around 75 percent is marketed through live bird marketing channels.

Because they use the “live in, dead out” system, the risk of slaughterhouses transmitting the HPAI virus is ranked as very low[10]. The risk to the environment could be limited only to absence of treatment facilities in the drainage systems or if there is poor C&D during processing, lack of decontamination of bird vehicles and crates, and improper transporting of waste products.

[10] Dent *et al.* (2011) considered slaughterhouses as potential agents of spread of HPAI among commercial farms in the United Kingdom.

Feed mills

Poultry production in Egypt depends on imported maize and soya meal. In addition, the main ingredients of any poultry feed include wheat bran, gluten, fishmeal, concentrates, premix, mono-calcium-phosphate and salt. The total annual poultry feed requirement is around three million tonnes (Engelen 2011). Most large-scale poultry companies in sectors 1 and 2 mill their own stock feed, while commercial production farms in sectors 3 and 4 rely on large feed mills via feed suppliers who receive a set amount of feed on credit, which they then pass on to poultry farmers on credit basis. The total number of feed plants in 2005 was estimated at 323 producing about 499 000 tonnes of feed composed of 313 200 tonnes of starter feed, 96 100 tonnes of finishing feed and 89 900 tonnes of layers feed (Ibrahim *et al.* 2006).

Published data about feed mills are limited and of poor quality. Large feed mills may be located out of the Delta region and distribute their products all over the country, so feed may transported for distances of over 800 kilometres. The small feed mills, which are located near to poultry farms, deal directly with their farmer clients and may produce feed according to special formulae requested by farmers.

Feed mills play a supportive role in the development of the poultry industry through provision of good quality feed, credit and advice. Risks related to feed mills could be contamination of feed ingredients by droppings of wild birds and/or mycotoxins due to low quality or improper storage of ingredients. The temperatures in heaps, in addition to the temperature and pressure conditions involved in processing, are sufficient to inactivate the virus (Kasemsuman *et al.* 2009). Thus, the possibility of release is very low, exposure is high and the risk can be considered as very low; if release occurs, the impact will be medium due to the dilution effect of contaminated droppings, so the overall risk estimate will be low with high uncertainty.

A further risk could be associated with the high frequency of movement of vehicles and trucks that deliver feed to farms through circulation among farms of different species, types of production, ages and locations on a daily basis, with no vehicle C&D or change of clothing/footwear at farm gate or mill level. Feed vehicles visit one or two farms a day, often without wheel dips (85 percent), with vehicles parked close to poultry houses (78 percent) or without the provision of clean clothing for visitors (88 percent). This situation potentially increases the risk of virus circulation among farms (Ali *et al.* 2013a).

The probability of release is high and exposure is considered medium due to indirect contamination (vehicles and drivers) and indirect transmission and high frequency of movements among farms. The impact could be considered medium due to silent disease (low viral load) transmission among vaccinated flocks, different production types (broilers, layers and breeders), species (duck, chicken and turkey) and locations. Hence, the overall risk estimate for feed mills in the poultry value chain is considered medium.

A critical practice is movement of drivers and vehicles without C&D at farm or mill gates.

MOVEMENT CONTROL ALONG THE POULTRY VALUE CHAIN

Outbreaks of HPAI in poultry need to be controlled as quickly as possible, both to prevent human cases and to protect the poultry industry. Strict movement control and tracing are important control measures during outbreaks, including forward tracing of contacts of an infected farm in order to respond quickly to secondary infections. Knowing when a virus has been introduced into an infected flock is essential for optimised tracing because it permits focusing on contacts between the time of virus introduction and culling. However, this requires both identifying the contact that infected the flock (backward tracing), which is often unknown, and how HPAI is transmitted in poultry flocks (Bos *et al.* 2007). In Egypt, lack of application of biosecurity programmes on farms, lack of disease notification, absence of movement control for poultry and their products among different districts and governorates with high poultry-dense areas, as well as weak capacity building capacity in the veterinary public health sector were and still are the most important contributing risk factors for the spread of HPAI and human infection (Kaoud 2007; FAO 2009a).

At commercial farm level

All short and long cycle farms (exotic broiler, layer, breeder, *Shamourt* or *Baladi* turkey, duck flocks) in Egypt must be tested for H5N1 infection before birds are sold. The village or district level veterinary authority collects tracheal and cloacal swabs, blood and litter samples, and sends them to authorised laboratories. The cost of inspection and test is paid by farm owners (about EGP 250 for 5 000 birds and EGP 100 for the litter test). In the event of negative results through real-time PCR (RT-PCR) the owner pays EGP 20 for each 800 birds (one vehicle load) to receive a transport permit for taking them to the slaughterhouse; farm owners offer the transport permit to bird collectors who may or may not purchase it. The slaughterhouse signs and stamps a detachable part of the transport permit indicating that the birds have been received. The farm owner is responsible for returning this part to the authorities. For litter movement, another certificate is issued by the district authorities. In the event of positive results, the birds are condemned, regardless of whether the flock has been vaccinated or not, or shows clinical signs or high mortality or not. Only registered and poultry association member farms receive compensation. It is important to note that the pre-slaughter certification does not include serological results which could be useful for producers to evaluate the efficacy of their vaccination programmes. Collected serum samples are discarded and not tested by authorised laboratories except in the case of positive PCR results (Peyre 2011), raising a question about which decisions are taken if a farm has a positive high serological titre and a positive PCR test result.

Governorate-level and district level adherence to restrictions, flock testing and certification prior to sale/movement to slaughterhouses varies widely (Peyre 2011 and personal communication with different farm owners from different locations). Many birds reach the slaughterhouse only on paper; and they end up in LBMs and poultry shops in towns (as indicated by a few bird collectors who asked farm owners for the slaughtering permission certificate). Compliance with certification for poultry transportation is generally sub-optimal, because only registered farms (<20 percent of the farms) seek such services (EMPRES/FAO-GLEWS 2010). This is indicated by the total number of farms subjected to pre-slaughter testing. In 2010, the figure was 33 086 and in 2011, after the Egyptian revolution of 25 January, the number of monthly tested pre-slaughter farm samples had fallen to 40 percent of the level of the same period the year before[11].

Commercial farms follow their own vaccination protocols (including vaccine type, vaccination doses, etc.), indicating the lack of clear mandatory vaccination programme at governorate level. At the same time, the official veterinary services only visit farms for bird sampling and/or flock culling, which has resulted in a lack of trust in and poor cooperation with official veterinary services by farmers. In addition, due to the absence of a real compensation programme, disease reporting and notification is poor.

RT-PCR test results and movement permits could be cross-matched with slaughterhouse information to assess how many of the birds allowed to be transported off the farms actually reach the slaughterhouses and how many enter LBMs, and could be used to create a database on volumes, directions and timing of bird movements for developing a tracking and tracing system (Engelen 2011).

Most sector 2 and 3 producers buy feed on credit basis until batch selling, and fear of loss may lead them to not notify, to illegally sell infected birds and to hide and dispose of dead birds in improper ways.

At bird collectors/traders level

All bird collectors/traders now have to pass the 'Poultry Boursa where they obtain permits indicating the price ("karta") before proceeding with the selling process. The permit is locally referred to as "karta", and is often issued by the governorate veterinary services. The 'karta' is a necessary document that allows the traders to pass through the various road check points. Any vehicle transporting poultry without a "karta" is fined EGP 1 000, of which 20 percent goes to the police department, 40 percent to the local government and remaining 40 percent to the poultry Borsa.

Traders rely on their mobile phones to know from where they could get birds, farm gate price and where active police check points are located. They have a network of buyers and receive information from brokers. The price indicated by brokers is the one accepted. Most traders avoid road check points, to reach to points where they offloading birds either at poultry shops, LBMs and other buyers such as slaughterhouses. In the past, government agents attempted to control movements of birds through various mechanisms and ensure their arrival at the designated destination but with limited success. There is no systematic washing and disinfection of crates and vehicles (Engelen 2011). Some traders purchase birds known to be infected at a very low price and resell them via door-to-door peddlers, or to a slaughterhouse, which, in turn, sell frozen products to fast food outlets. Buyers, such as most villager women can inadvertently facilitate such cheaper trades, due partly to their low level awareness of the associated risk. This type of practices enhance virus seeding and spread in villages. Most collectors work individually and do not belong to recognised companies. They are not registered, hence not under the supervision of veterinary authorities. They have no official training and are ignorant of good practices, biosecurity measures, zoonotic disease and health risks, etc.

[11] Personal communication with Dr Mohamed Khalifa, NLQP

There is a need to control bird movements and enforce hygiene and environmental measures. A system of registration of bird traders should be established and mandated to a competent public sector organization. The later ratify and implement regulation on C&D of vehicles and crates, without which any bird or by product transport permit should not be issues. The system need to gradually control the movement of birds to poultry shops and LBMs.

The Borsa "karta" could also be used as an indicator of how many animals have been transported from where to where, and used for bird tracking and tracing.

At poultry markets

There are around 15 892 retail shops in Egypt that sell either live or freshly slaughtered birds to consumers. In addition, 4 305 small slaughtering and de-feathering points exist that sell freshly slaughtered and chilled birds and bird parts (Hosny 2006). LBMs are key links between the commercial and small-scale household poultry sectors. Egypt has recently implemented bans on selling live birds in open markets (Law 70/2009, MoALR MD 941/09), but the ban has had little effect and LBMs continue to operate. Some governorates are enforcing decrees related to the banning of unregistered poultry farms and control of bird movements. Enforcement varies from one governorate to another, but is generally weak (EMPRES/FAO-GLEWS 2010).

Multiple LBMs are totally unregulated, and have no animal health inspection, return unsold live birds, no cleaning and disinfection facility, no separation between birds for consumption and rearing, no separation of species, no mandatory application of "live in, dead out", and no registration of traders nor sellers. The latter means that no certification is required for poultry to enter LBMs/poultry shops, that no biosecurity measures are enforced or applied by individuals and that there is no bird tracing.

In slaughterhouses

Although these plants all have a resident veterinarian inspector in name, the current situation presents no evidence that such inspectors are effective for protecting public health, preventing hazardous situations or ensuring that slaughtered birds comply with all the requirements related to testing for HPAI, etc. (Engelen 2011). In most cases, the surroundings are dirty, there is no sign of rodent control, and cats live close by. Most have no provisions for collecting and processing blood, and intestines and feathers are collected by what is called a "cooker". However, there is no certainty that dangerous offal is being processed in a responsible way (Engelen 2011). Only the automatic slaughterhouses are better designed to comply with GMP and HACCP requirements.

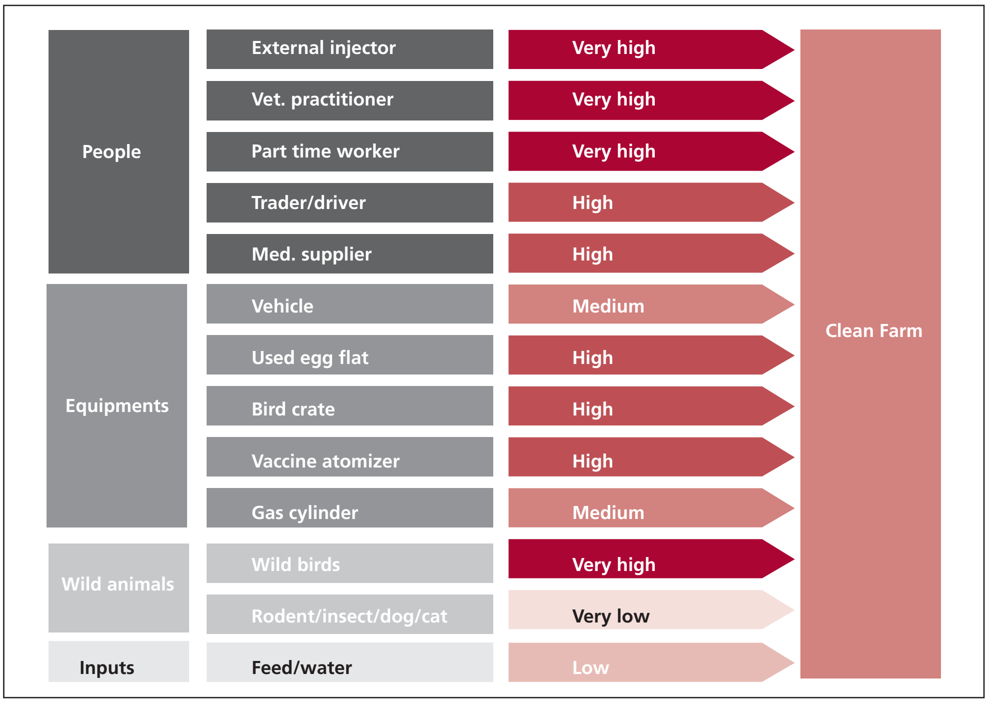
Certification and traceability can promote virtuous quality cycles that combine risk reduction with higher product value and incomes along supply chains. So the weak capacity which is reflected by a poorly certified supply chain, lack of bird tracing systems, and registration of intermediaries, traders, pedlars, transporters and retailers, in addition to minimal, if any, veterinary inspection of traditional LBMs, facilitate movements of diseased birds and low quality birds without recognition.

Conclusion and Recommendations

Risk analysis can assist in selecting risk mitigation measures to be applied in a prevention strategy plan against H5N1 HPAI (Goutard *et al.* 2007; Martínez *et al.* 2007; Sabirovic *et al.* 2007). In this sense, the risk assessment framework can help in identifying at-risk nodes, which is useful to optimise targeted surveillance (Martínez *et al.* 2007). Movement of people, birds, vehicles and/or equipment among farms, household production systems, slaughterhouses, hatcheries, feed mills, live bird markets and bird collection points are the main causes of disease transmission and are present in all risk pathways in the poultry value chain.

For commercial farms, the risk associated with movement of people is considered highly significant due to weak farm gate decontamination activities, and could be separated into very high risk (with low uncertainty associated with external injectors, part-time day farm workers, visiting veterinary practitioners) and high risk with feed delivery, egg-collecting and litter collecting drivers, while medical representatives and drug suppliers represent medium risk. Feed, egg and litter vehicles represent medium risk with high uncertainty. The risk associated with sharing equipment among different farms is high with medium uncertainty for used egg cartons, vaccine atomizers and bird crates in the case of multi-age farms, followed by low and medium risk associated with bird crates for one-age farm and gas cylinders respectively, with medium uncertainty. The overall risk for the key pathways was very low for rodents, insects, dogs and cats, and very high for wild birds. Feed and water inputs represent low risk. For a graphic representation of risk levels for different categories, see Fig. 7 below.

Figure 7. Overview of risk estimates for different disease carriers into commercial farms



For small-scale household production, the risk associated with trading of adult waterfowl, *Baladi* chicken, non-resident commercial farm workers and wild birds that share flock houses, feed and water is considered very high. The overall risk associated with purchased young birds and exotic chicken is low and medium, respectively (Fig. 8).

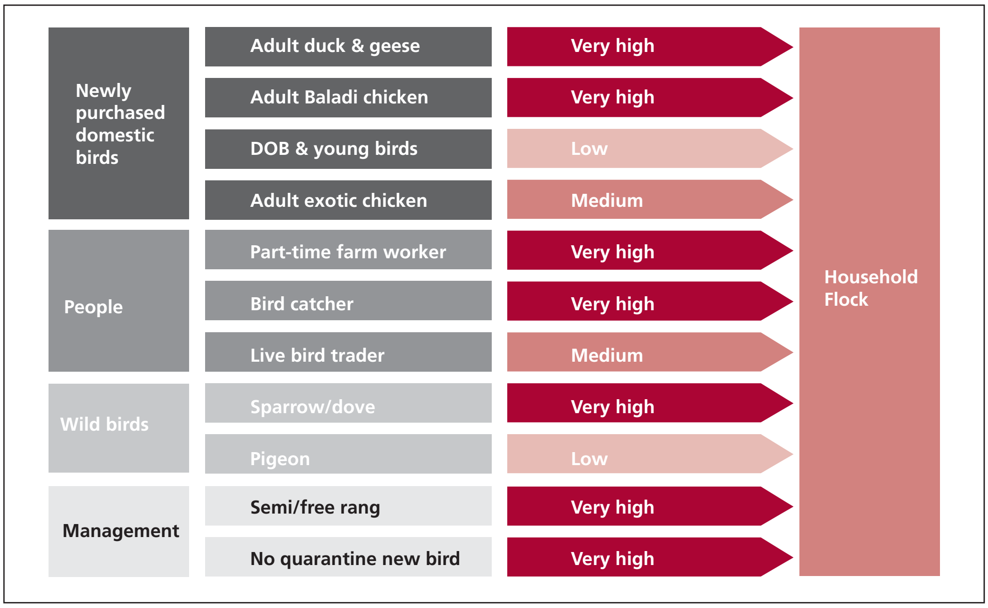
Application of a "live in, dead out" policy, C&D, and live trading of adult waterfowl and *Baladi* chicken by LBMs do not represent a very high risk for small-scale household production system.

Litter collection points, feed mills and slaughterhouses represent very low risk for the poultry value chain because they do not facilitate virus replication or shedding. However, they do represent risk through the movement of vehicles and drivers with poor biosecurity measures among different farms and locations.

Most sector 2 and sector 3 producers buy feed on credit basis until batch selling and the fear of loss may lead them not to notify in case of infection, illegally sell infected birds and hide and dispose of dead birds in improper ways. Some specialised traders actually profit from the disease by purchasing birds known to be infected at very low prices and reselling them without this information via door-to-door peddlers or to slaughterhouses which, in turn sell frozen birds to fast food outlets. Buyers such as villager women and/or some fast food retailers with no or low level of risk awareness facilitate this type of cheaper trade and disease spread by trying to save money.

The absence of disease signs in some duck species led Kim *et al.* (2009) to suggest the concept of ducks as "Trojan horses" of H5N1 in their surreptitious spread of virus. In Egypt, many Trojan horses for H5N1 are in place and include poor farm-gate biosecurity measures, widespread and inappropriate H5 vaccination protocols, co-circulation with H9N2 and unregulated live bird trading facilitate "silent spreading" of H5N1 HPAI viruses, continuing the circulation and endemicity of the disease.

Figure 8. Overview of risk estimates for different disease carriers into small-scale household poultry production systems



As long as birds are reared under management systems with poor biosecurity, including free movement without inspection or tracing, they represent a risk that H5N1 HPAI will continue to spread.

Thus critical control points for prevention of avian influenza virus transmission along the poultry value chain include strict farm gate biosecurity system by commercial producers, increased awareness of the importance of placing newly-purchased birds in quarantine and keeping birds in a confined environment in small-scale household production systems, followed by strict application of a "live in, slaughtered out" policy by LBMs, restructuring of LBMs in such a way as to permit sound decontamination, directional flow from dirty to clean zones, and efficient movement control by regulatory authorities which requires strong capacity building.

Due to the high density of poultry farms and small-scale household production in most governorates, there is a need for national standard producer guides designed to regulate and support the application of good management and biosecurity systems by poultry production and trade sectors and actors involved in the poultry value chain, with clear critical objectives that must be met. Programmes should be based on clear, scientifically justified principles suitable for the Egyptian situation that detail a range of measures that are applicable to all levels of poultry production and auditable measures intended to prevent disease-causing agents from entering and/or leaving premises. Formation of regional groups or production type associations or committees may serve as a tool for applying social pressure for disease mitigation and assisting the authorities in the implementation and improvement of monitoring, coordination, communication, transparency and agreement among the industry actors. Responsibility for monitoring and controlling poultry diseases, regulating the market, preventing the industry from dramatic overproduction losses and making the poultry sector work for all are shared responsibilities of the public and private sectors.

It is critically important to improve risk management along market chains. Therefore, it is necessary to improve farm biosecurity, and review and amend current policies and practices related to the marketing of live birds, taking into account the capacity to enforce decisions. Efforts to limit the spread of the virus by raising biosecurity levels, and controlling the movement of birds, poultry products and by-products should continue, along with the use of certification as a tool to achieve this. Movement control for household poultry also requires a tailored approach to minimise the impact of such measures on people's livelihoods. Finally, efforts should be made to ensure the proper disposal of waste.

There are a number of factors that increase and decrease risk for disease introduction. These determinants can be classified as either internal factors related to the farm e.g. farm design, structure and activities, or external factors, e.g. lack of supporting training centers or reference guide for good management practice.

Table 9 indicates the risk factors associated with different node levels and related actions designed to reduce (if not eliminate) these risks.

Table 9. Factors affecting risks at different nodes along the poultry value chain

	Factors increasing risk	Action to reduce risk
1. Commercial farms		
Internal factors	Weak traffic control	- Restriction of unnecessary human traffic (a major component of a sound programme for disease prevention) - Use only service crews (for broiler/egg pickup, feed delivery and other operations) who comply with strict sanitary measures
	Farm owners, workers and drivers of most Sectors 2 and 3 farms lack awareness and training on basic principles of disease transmission and prevention or control	Maintain an ongoing worker education program on biosecurity and the risks of disease introduction
	Farm workers assigned to serve several poultry houses	- Restrict access to poultry areas to essential workers only - Different poultry houses should be staffed separately
	Weak or poor C&D facilities and infrastructure in most sector 2 and 3 farms	- Personal hygiene procedures must include clear and precise information - Use clothing that belongs to the farm and can be easily cleaned and disinfected - Footwear used inside the farm should belong to the farm and be used after thorough cleaning and disinfection; simple footwear can be used, such as flip-flops or boots, but they must be fully cleanable - All workers and visitors must wash with soap then put on disinfected footwear before entering the farm
	High personnel turn-over in commercial poultry farms	- High turnover rates among workers, constitutes a big constraint against sustainable implementation - Workers benefits and rewards should be considered as a part of biosecurity cost
	Overreliance on vaccination and relaxation of biosecurity measures	Delivery of developed messages, emphasising that biosecurity must come before vaccination, that biosecurity reduces the exposure to and infection from not only avian influenza but most poultry pathogens, while vaccination only reduces and does not prevent susceptibility to infection, does not prevent virus shedding and complicates detection by masking clinical signs
	No or poor C&D for vehicles	- Where possible, vehicles (apart from essential vehicles) should not be allowed to enter the premises - Perform C&D of vehicles in the dirty zone - Allow personnel, vehicles and equipment to enter farms only after C&D - Drivers not permitted to leave the vehicle cabs but if this is necessary, they should wear overshoes or change their shoes to farm shoes; in addition, drivers should not enter poultry houses or come into contact with poultry and should avoid faecal contamination of clothing and footwear - Construction of stores that are easily accessible and used without the need for external people/vehicles to enter the farm could lower the level of risk

	Factors increasing risk	Action to reduce risk
Internal factors	The practice of exchange of equipment between farms and farmhouses that may be contaminated and spread infectious agents	- All equipment and materials that enter the poultry farm, regardless of size or use, must be decontaminated in the dirty zone on entry and in the buffer zone on exit; all equipment and materials must be visibly free of organic matter accumulations to reduce the risk of disease transmission - Do not allow entry of used paper egg flats - Poultry crates should be cleaned and disinfected in a washing station, and should be visibly clean before being allowed to enter the farm; it is preferable not to allow the entry of wooden crates because of the difficulty of cleaning and disinfecting them - Prevent the sharing of equipment with other farms
	Improper disposal of dead bird and farm waste	Properly dispose of dead birds either by composting, burying or incineration
	Broken windows, spilled feed, farm trees, stagnant water and caked litter attract wild birds, insects and rodents to farms	- Regularly check and repair wire screening on the sides of the poultry house and feed store to prevent wild bird access - Promptly clean up spilled feed - Avoid presence of stagnant water, ponds and trees on the farm - All ponds around poultry houses should be drained
	Litter piled up in an open area without any cover or compost	- Promptly compost caked litter - Apply insect control program
	Unsecured feed storage facilities	- Secure all feed storage areas, clean up spilled feed and manure, and regularly sanitise buffer zones to minimise rodent infestation and attraction of wild birds
	Presence of piles of old equipment and construction material abandoned near the poultry houses provide shelter and hiding place for rodents	- Do not keep piles of old materials on the farm - Maintain a clean 2-metre wide weed-free zone around building foundations and concrete foundations to discourage rodent burrowing/tunnelling under buildings
	Use of untreated water for drinking	Use water disinfectants at recommended dilution levels, application rates and contact times
	Underreporting of outbreaks	Promote the compensation program among producers to encourage farmers for reporting
External factors	Lack of a national, regional or local body to oversee and take necessary steps to improve and regulate movement of poultry and poultry products	- Encourage formation of poultry associations or committees to make the poultry sector work for all - Treat all sector 3 farms located in the same district or region as one unit, applying an "all in, all out" policy, keeping just one type of bird, synchronising production and marketing activities, and applying area biosecurity
	most poultry farms are not registered. Most farms also operate on rental basis. The rental period is commonly a year and renters focus on maximising their benefits during this limited period. They have no desire to spend money on long-term structures or any other investment to enhance biosecurity	Support the use an in-built gradually expanding set of verifiable biosecurity requirements of a simplified poultry farm registration system
	No extension or farmer-focused training on good poultry management practices	Encourage formation of poultry management and biosecurity training centres by universities and qualified institutes

Factors increasing risk	Action to reduce risk
Lack of national programmes for biosecurity implementation at all levels of the poultry value chain	Establishment of regular training programmes on sound biosecurity measures and their application for different stakeholders (farmers, service crews, transporters, etc.)
Lack of national standard guides designed to support the development of farm-specific biosecurity protocols for different sectors	- Development of a national guide - Improve access to information through training and financial resources and micro-credit to build human and farm resource capacity
Unregulated vaccine use by commercial farms and lack of evaluation or monitoring of the efficiency of vaccination programmes	Regularly monitor different influenza vaccines and programmes for their efficiency in protecting flocks
2. Small-scale household production sector	
Purchasing birds from unknown new supplier	Purchase birds especially adult ones from a trusted supplier
Introduction of new birds to flocks without a quarantine period	Quarantine newly purchased birds for at least 10 days before mixing with house flock
- Free and semi-range scavenging bird production - Sharing of feed and water with wild birds	Keep birds indoors in a confined environment
Lack of specific clothing and footwear for bird house	Make available specific clothing and footwear for bird house
Improper bird disposal (throwing on roads or watercourses)	Dead birds should be removed promptly so that they do not become a source of infection for the rest. Dispose of them by burying, incinerating or composting. Throw to pets, in water canal or waste land contaminate the environment and help circulation of pathogens in the surrounding area by wild birds, insects and pets and returned back to your house
Mixing different species and ages	- Separate between different species , as one species may carry and be immune to a disease that is infectious for another - Do not mix different ages, as older ages are immune, less susceptible and may carry infectious organisms for young birds that have not yet developed immunity
Lack of notification in case of disease outbreak	Visibly sick and moribund birds should be separated immediately from the healthy ones in order to limit the spread of the disease. If a disease is suspected, either consult with a veterinarian or send a few sick birds to a village veterinary department for diagnosis
3. Live bird markets	
Mixing of different species	Spatial or temporal separation between birds for rearing and birds for consumption, and among birds of different species and different ages should considered
Unsold birds returning alive to their flocks	- Apply a “live-in, dead out” system for adult birds - Sell all birds entering the market within the working day; birds remaining should be slaughtered at the end of the working day and kept frozen for the next day
- Absence of cleaning facilities in the market area - Market places that are unsuitable for cleaning (sand or mud)	Create a specific area in LBMs for cleaning activities

Factors increasing risk	Action to reduce risk
Lack of inspection of birds before entry and no tracking system at market level	LBMs should be authorised and placed under veterinary supervision
4. Litter collection points	
No strict adherence to hygienic measures during and after litter management by either farm and collection point workers	- Strictly adhere to hygienic measures during and after litter management - Pile litter up for a few days or compost before transporting it out of the farm premises
Movement of vehicles among different farms and locations without C&D practices at farm or collection point levels (which clearly presents opportunities for the dissemination of AI viruses to poultry producers).	- Only allow personnel and vehicles to enter farm after C & D - Drivers must not be permitted to leave the vehicle cabs
5. Hatcheries	
Circulation of used egg cartons between hatcheries and producing farms	Do not permit circulation of used paper egg flats
Lack of knowledge of good production practices	- Keep no mixing of eggs and birds - Keep the workers' flow from the clean zone (egg setter) to the dirty zone (hatching unit), with foot dips at the entry, hand-washing facilities, showers and protective clothing facilities - Allow personnel to enter hatchery only after clear personal hygiene procedures
No or poor C&D of egg and bird vehicles	- Where possible, vehicles (apart from essential vehicles) should not be allowed to enter the premises - Perform C&D of vehicles in the dirty zone - Drivers should not be permitted to leave the vehicle cabs but if this is necessary, they should wear overshoes or change their shoes to farm shoes
- Lack of knowledge among workers about main sources/carriers of disease infection - Lack of awareness of basic principles of disease prevention among hatchery workers and drivers	Maintain an ongoing worker education program on biosecurity and the risks of disease introduction
Lack of national standard biosecurity and management guides/SOPs designed to support hatcheries	- Design standard guides and SOPs to support hatchery-specific biosecurity systems - Establish national programmes of biosecurity implementation at hatchery level - Encourage the development of hatchery associations to assist the authorities in hygiene and safety improvements
6. Slaughterhouses	
No or poor C&D of bird crates and vehicles	Strict C&D of vehicles and crates before leaving the premises
Lack of knowledge of the basic principles of C&D	- Maintain an ongoing worker education program on biosecurity and food safety - Encourage development of slaughterhouse associations to assist the authorities in hygiene and safety improvements - Establish national pro grammes of biosecurity implementation at slaughterhouse level
Lack of national standard biosecurity guides / SOPs designed to support all types of slaughterhouses	Design standard biosecurity and management guides / SOPs to support manual and semi-automatic slaughterhouse activities

Limitations of the study

The main limitation of this study is poor or absence of data that would help in estimating and evaluating levels of likelihood, impact and risk. These include:

- data on transmission routes responsible for different outbreaks;
- updated data on numbers of different poultry types/species produced by each sector;
- updated information on the HPAI situation in wild birds and their role in HPAI epidemiology; frequency and rate of contact between wild and domestic birds;
- epidemiological information or studies on relations between HPAI isolates of commercial farms, LBMs, household birds and wild birds;
- data on litter collection sites, number and capacity of these collection points, average storage time for litter batches, the seasonality of maximum activity of these points, H5N1 virus prevalence or its viability in these points, and the status of the H5N1 virus in wild birds feeding on litter;
- data on isolates submitted to the GeneBank[12], that would include information on types of production (breeder, layer or broiler), breed and vaccination status.

[12] GeneBank is the National Institutes of Health (NIH) genetic sequence database, an annotated collection of all publicly available DNA sequences. See http://www.ncbi.nlm.nih.gov/genbank/.

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