

Study on the presence of highly pathogenic avian influenza (HPAI) virus and Newcastle disease virus in live bird markets in Tanta District, Gharbia Governorate, Egypt



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RECOMMENDED CITATION

FAO. 2009. Study on the presence of highly pathogenic avian influenza (HPAI) virus and Newcastle disease virus in live bird markets in Tanta District, Gharbia Governorate, Egypt. Prepared by Aly M. Mona, Samaha, H.A., Galal, S.A., Arafa A., Zahra Ahmed and Karin Schwabenbauer. *AHBL - Promoting strategies for prevention and control of HPAI*. Rome.

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Contents

TABLES	1
INTRODUCTION	2
MATERIAL AND METHODS	3
SAMPLING METHODS FOR BIRDS	3
LABORATORY PROCEDURES FOR DIFFERENT PURPOSES	4
COMPOSITION OF THE FIELD TEAMS	4
GATHERING AND ANALYSIS OF THE QUESTIONNAIRES FROM THE MARKETS	5
QUALITY CONTROL	5
RESULTS	5
DESCRIPTION OF THE MARKET/TRADERS	5
LABORATORY RESULTS	7
DISCUSSION	8
CONCLUSIONS AND RECOMMENDATIONS	9
ANNEXES	10
ANNEX 1	10
ANNEX 2	13
ANNEX 3	14

Tables/Figures

TABLE 1. LIVE BIRD MARKETS IN TANTA DISTRICT, GHARBIA GOVERNORATE	10
TABLE 2. THE TABULATED DATA OF THE SURVEYED MARKETS AT TANTA DISTRICTS GHARBIA GOVERNORATE	14
TABLE 3. RESULTS OF AI DETECTION IN LIVE BIRD MARKETS SURVEILLANCE USING RRT-PCR AND ISOLATION IN TANTA DISTRICT, GHARBIA GOVERNORATE, 16–27 MARCH 2008 (WEEK 1)	15
TABLE 4. RESULTS OF AI DETECTION OF LBM SURVEILLANCE USING RRT-PCR AND ISOLATION, TANTA DISTRICT, EL-GHARBIA GOVERNORATE, 16–27 MARCH 2008 (WEEK 2)	16
TABLE 5. RESULTS OF AI DETECTION OF LPM SURVEILLANCE USING ELISA AND	17
TABLE 6. RESULTS OF AI ANTIBODY DETECTION IN LIVE POULTRY MARKET (LPM) SURVEILLANCE USING ELISA AND HI TESTS, TANTA DISTRICT, EL-GHARBIA GOVERNORATE; 16-27 MARCH 2008 (WEEK 2)	18
TABLE 7. RESULTS OF ND DETECTION IN LIVE POULTRY MARKETS (LPMs) SURVEILLANCE USING RRT AND HI TESTS, TANTA DISTRICT, EL-GHARBIA, GOVERNORATE, 16–27 MARCH 2008 (WEEK1)	19
TABLE 8. RESULTS OF ND DETECTION SURVEILLANCE IN LPM USING RRT-PCR AND HI TESTS, TANTA DISTRICT, EL-GHARBIA GOVERNORATE, 16-27 MARCH 2008 (WEEK 2)	20
TABLE 9. NO. OF SAMPLES AND THEIR SOURCES, AND NO. OF POOLED SAMPLES (WEEK 1)	20
FIGURE 1: RATIO OF CHICKEN TO DUCKS AND GESE IN THE SELECTED MARKETS	10
FIGURE 2. MAP OF EGYPT WITH GHARBIA GOVERNORATE	11
FIGURE 3. GHARBIA GOVERNORATE AND ITS DISTRICTS	11



Introduction

Gharbia Governorate is one of the Egyptian production centres for ducks and chickens in the Delta, providing poultry for the whole region and probably beyond. In 2006, a total of 1 067 outbreaks of highly pathogenic avian influenza (HPAI) H5N1 infections were recorded in Egypt, of which 59 cases were in Gharbia Governorate, which included 53 farm and six backyard outbreaks. In 2007, Gharbia Governorate had the highest incidence of HPAI H5N1 among Egypt Governorates, with 28 out of 282 recorded outbreaks, which included two farm and 26 backyard outbreaks. From January until March 2008, 20 HPAI H5N1 outbreaks were recorded in Gharbia Governorate, two farm and 18 backyard outbreaks. The 20 outbreaks were in six districts, Mahala, Zefta, Tanta, Kafr Elzayat, Kotoor and Samanood. Tanta had four outbreaks of H5N1 AI in four villages, Fesha Sleem, Meet sodan, Desheet and Shobar.

In order to better understand the epidemiology and ecology of the disease in Egypt and to develop an appropriate strategy sensible to smallholder and poultry breeds, a survey of avian influenza (AI) virus presence in live bird markets (LBMs) in Tanta, Gharbia, was proposed. The hypothesis is that due to the importance of LBMs in the trade of poultry in the country, the survey will detect virus presence in the poultry population at the markets there. LBMs are the main gathering points for poultry, where all kinds of poultry species are mixed, coming from different types of sources (farms, backyards, different districts and even governorates). If HPAI virus is present in farms or backyards, it should also be found at LBMs, which may play a decisive role in the spread of the disease. Since poultry is also slaughtered at the markets, the risk of human infections is not excluded.

The survey was done in March 2008 during the main market days and repeated twice within two successive weeks. Samples were collected and examined for HPAI virus and Newcastle disease (ND) virus from chickens and ducks, and internal organs samples were kept for later analysis of duck plague, since these diseases are the most important differential diagnosis for clinical HPAI. This survey was part of the AHBL Project,¹ aiming at developing smallholder-sensitive policies and strategies for the control of HPAI, based on pilot studies. It was conducted by the General Organization for Veterinary Services (GOVS) at the governorate and central levels, and the National Laboratory for Veterinary Quality Control on Poultry Production (NLQP), with inputs from the Food and Agriculture Organization of the United Nations (FAO).

¹ The project "Promoting strategies for prevention and control of HPAI that focus on smallholder livelihoods and biodiversity" (GCP/INT/010/GER), covers Animal Health, Breeds and Livelihoods (AHBL).



Material and methods

Market selection Maps of all LBMs in Tanta District were available at GOVS. There are 24 markets in Tanta District (Annex 1, Table 1). The selection of the markets for the survey was done by GOVS, based on the data available and considering the criterion of the ratio between chicken and ducks in those markets. Villages with a local market and following additional criteria were selected for the survey (Annex 1, Figure 2a and b):

Villages with an equal number of chicken and ducks;
Villages with more ducks than chickens;
Villages with more chickens than ducks.

A total of ten markets were selected for the survey (Annex 1, Table 1)

Poultry population and species of the sampled markets are provided in the Annex (Annex 1, Table 1). As per the design of the study, each market was twice surveyed during the “poultry day” with an interval of one week. On each working day, two markets were surveyed by two teams working in parallel. Concurrent to the sampling of birds, background information on the market condition and biosafety measures were collected using an FAO prepared questionnaire (Annex 2).

Sampling methods for birds

Sampling plan The draft sampling plan was prepared by NLQP and discussed jointly with GOVS, then reviewed during a follow-up meeting organized by FAO. As per the agreed plan, 720 samples were to be collected (50 percent ducks and 50 percent chicken), as described below.

Training A one-day workshop was organized jointly by GOVS and NLQP in collaboration with FAO. It was conducted locally in the training centre at Tanta District. Participants were 51 veterinarians from the GOVS at the central and governorate level and from the regional laboratory of the Animal Health Research Institute. The training given by two trainers from the NLQP aimed at demonstrating the proper sampling methods and describing the different types of samples that needed to be collected from the LBMs, and the proper identification, preservation and transportation of the sampled birds. Materials needed for sampling were prepared centrally by both GOVS and NLQP staff, and were sent to Tanta District prior to the field survey.

Sampling procedure The sampling of the live birds was carried out from 16 to 27 March 2008. Ten LBMs were visited and 713 live birds were bought for sampling using the agreed sampling protocol (Annex, Tables 9 and 10). Different types of samples were collected for different purposes: blood samples from chickens and ducks were collected for serological tests; tracheal and cloacal swabs were collected for detection of AI virus and ND virus using real time PCR; and the same samples were used for virus isolation. Further, the internal organs of ducks were kept in a freezer at -80°C for further investigations concerning duck viral enteritis.



By approaching the market early in the morning, the teams observed the market for any diseased birds showing any clinical signs, such as respiratory and nervous symptoms, cyanosis in comb and wattles, and diarrhoea. The presence of any of these signs was used to select the bird for sampling. In case there were no diseased birds, healthy birds representing the different categories of age, species and sources were selected randomly.

Sampling preparation took place at Tanta Regional Laboratory and then samples were transported to the National Laboratory in Cairo for further analysis.

Laboratory procedures for different purposes

The procedure for pooling of individual samples for AI and ND detection using QRT-PCR was as follows: Cloacal and tracheal swabs were pooled separately and the examined pools were a mix of both. This was done centrally in NLQP with samples from five birds together in one pool, according to the following criteria:

Species (chicken pools were separated from the duck pools)

Health status, (diseased birds were pooled separately)

Source (traders or village)

Individual tracheal and cloacal swab samples were not kept for further investigations.

Procedure for real time PCR for detection of AI The method employed was according to Veterinary Laboratories Agency (VLA), using a one-step real time PCR kit; primers and probes were designed according to VLA sequences and the test was conducted with a Stratagene MX3005P real time PCR machine.

Procedure for real time PCR for detection of ND The method used was according to the VLA one-step real time PCR Kit Qiagen. Primers and probes were designed according to VLA sequences and the test was conducted with a Stratagene MX3005P real time PCR machine.

Procedure for AB detection of AI and ND ELISA test: detection of AI antibodies by using commercial kits (Biocheck, Netherlands) for chicken sera and (ID VET, France) for duck sera.

Haemagglutination inhibition (HI) test Detection of both AI and ND antibodies was done according to the 2005 Protocol of the World Organization for Animal Health (OIE).

Composition of the field teams

The field teams surveying each market were composed of four members, two members from central GOVS and NLQP, and two from the local branch of GOVS in Tanta and the regional laboratory in Tanta, as well as one supervisor from GOVS. The central teams from GOVS and NLQP were permanent, while the teams from district were different. The GOVS members were responsible for interviewing the traders. Purchasing the birds was jointly conducted by GOVS and NLQ for each market, while sampling the tracheal, cloacal swab, blood samples and internal organs was done in the regional laboratory.



Gathering and analysis of the questionnaires from the markets

The questionnaires used contained qualitative and quantitative information on the markets, i.e. location, size of the market, periodicity, kinds of birds, other animals traded, slaughtering practices and provisions in place for disposal of carcasses and offal. Information on the sellers and the sampled birds was also collected by interviews and observations. The questionnaires used to collect data on the markets, the sellers and the sampled birds were entered into an Excel sheet, and analysed using SPSS (Annex 3). A total of 259 bird sellers were interviewed, of which 60 percent were sellers in small markets. The percentage of interviewees in mid-size and large markets amounted to 20 percent each out of the total.

Quality control

During the survey, a day of field visits was conducted by the National Project Coordinator (FAO), accompanied by a representative from the local branch of GOVS (Tanta Veterinary Office). The visit aimed to check the techniques for questionnaire administration and collection of laboratory samples. A post-survey operational meeting, attended by the field teams from both GOVS and NLQP, was carried out, and the findings and lesson learned discussed. The outcomes of these meetings will be used to improve field work performance in future surveys.

Results

Description of the market/traders

Location of the markets

The surveyed LBMs were located on streets in residential areas, most of which were in narrow or heavily crowded places where caged and free-range poultry (mainly ducks) were observed. Since the LBMs had been prohibited by legislation since 1997, all the markets are informal. This law was reinforced after the HPAI outbreaks. There is no veterinary inspection to protect human health (food safety) or to collect samples from sick or dead birds in the markets.

The market is distinctly subdivided into sections for trading vegetables and fruits, cereal and legumes, birds, eggs, and cheese. Clothes and kitchen utensils are sometimes sold as well. In addition, there are retail poultry shops and small slaughtering and de-feathering points in the markets.

Proportion of the different species/sources in the market

In all the surveyed markets, the sources of the traded birds in the three categories were farms and home productions from the different districts of Gharbia. However, the traded birds were not only from Gharbia Governorate, but also from surrounding governorates, namely Qualiobia and Menofia, and particularly in markets near neighbouring governorates.

The traded chickens were mainly from commercial farms and from different production systems. Most of the farm chickens for sale were broilers with a small number of layers at their end of production. Native breeds (*Balady*) chicken were mainly from home production and sold by women.



Most of the ducks, in all of the surveyed markets, were from home production. The numbers of sold ducks increases during feasts and Ramadan.

Ages of the birds sold

Birds of all ages were found in the surveyed markets, young birds usually for further rearing and restocking, and adult broilers, Balady and layers, for consumption. As opposed to chickens, day-old and young ducks and geese were rarely sold in the markets.

Poultry shops

Poultry shops sold poultry for consumption only, including commercial broilers, layers, ducks, turkey, pigeons and geese and the birds were kept until slaughter or selling within the shop. Traditional breeds were also sold in poultry shops. Birds were usually slaughtered at the shop and sometimes sold live.

Slaughter places, hygiene of the slaughtering, including disposal of offal

Birds were sold live and either brought home live or slaughtered in the market. There were two main types of small slaughtering and de-feathering points: the permanent type, which usually occupied the back of a small poultry shop, or a small enclosure; and the movable type, which followed the weekly village market. Most slaughtering took place at the market in the above-mentioned small-scale, unregulated slaughterhouses. The market infrastructure did not allow for safe disposal of the dead birds carcass and/or offal. At the end of the day, all garbage, offal, feathers and leftovers were collected and placed in a corner to be collected later by the municipality, on a daily basis. Children and young girls were often the ones who carry out this activity. Stray cats and dogs had free access to this waste.

The unsold birds

At the end of the market, the unsold birds usually had different trade channels.

The birds from home production usually traded by women were normally all sold. In a few cases, if there were unsold birds, women sold them to poultry shops and/or other peddlers for a lower price. Returning home with unsold birds was an uncommon practice among villagers, likely due to the pressing need for money.

The numbers of traded birds from farms was relatively higher than that of traded birds from home production. Consequently, the unsold birds were usually kept in remote storage areas, prepared for this purpose or used temporarily. These birds were usually re-introduced to another village market.

In the large LBMs, big cars were seen waiting in the market, collecting birds from villagers, women and peddlers. They usually delivered birds to other markets in the same governorate and/or to neighbouring governorates if there were price differences between the different markets. This practice allowed them to benefit from the already existing price differences.

Other species traded in the markets

In addition to the poultry, other types of animals such as rabbits, sheep, goats and cows were found in the surveyed markets. However, the number of these animals was considerably smaller than that of traded poultry.



Laboratory results

Results of detection of avian influenza in live bird markets

The samples were pooled by five birds. A total of 151 sample pools were examined, which included 73 pools in the first week and 78 pools in the second week. Cloacal and tracheal swabs were pooled together. Individual samples were not kept. Therefore, each positive pool reported here represents up to five positive birds.

Results of AI detection from LBMs in Tanta District in the first week (Annex, Table 5) showed a total number of 12 AI-positive pools using real time PCR against the M gene. Eight pools were made out of ducks samples and four out of chicken samples. These pools were recorded from four markets among the ten examined markets – Shony, Talbant Kayser (small markets), El Gaban (mid-size market) and Al Hekma (large market). Among the 12 positive AI pools, 11 of them were positive for H5 gene and all the 12 pools were negative with real time PCR against H7 gene.

Isolation of the virus from different markets that showed positive detection using real time PCR in the first week revealed isolation of four AI viruses from ducks in Shony, Talbant Kayser, El Gaban and Al Hekma markets. After one passage in SPF ECE, two AI viruses were isolated from chickens from Shony and Al Hekma markets, while isolation of AI virus failed for the Talabant Kaysar Market chickens.

In the second week (Annex, Table 4) AI in chickens was detected in one market out of ten using real time PCR against the M gene in Talabant Kaysar Market and was confirmed to be H5 when tested using real time PCR against H5 gene and was Negative against H7 gene.

Isolation of the AI H5 failed in this case after two blind passages in Specific Pathogen Free Embryonated Chicken Eggs (SPF ECE).

The market background data included the date, name of market and village, source and type of birds, and the nature of the market (formal or informal). This information was entered, reviewed and reported in an Excel spreadsheet.

Results of serological examination of HPAI in the live bird markets

Chicken serum samples were examined separately from duck samples and serum samples were tested individually.

Results of AI antibody (AI-Ab) detection from LBMs in Tanta District in the first week (Annex, Table 7) indicated that examined duck samples showed positive results for detection of AI-Ab with a percentage that ranged from 28.6 to 75 through the ELISA test. Examined chicken samples were positive for detection of AI-Ab with a percentage that ranged from 35.3 to 83.3 percent through the ELISA test.

The positive results of ELISA were confirmed with the HI test whenever there was enough serum to exclude the non specific result in ELISA. Positive ELISA samples from ducks were positive against AI antibody detection using HI test with a percentage ranging from 20 to 100. For chickens, samples tested positive with ELISA test were also positive for AI-Ab using HI test with a percentage ranging from 85.7 to 100. Results of AI-Ab detection from LBM in Tanta District in the second week (Annex 4, Table 8) indicated that examined duck samples showed positive results for detection of AI-AB with a percentage ranging from 23.8 to 43.7 through ELISA, except for duck samples in three markets, which were all negative. Tested chickens in one market were negative for AI-Ab detection and the rest of tested markets were positive for AI-Ab detection through the ELISA test, with a percentage ranging from 22.2 to



81. The positive results of ELISA were repeated with HI test whenever there was enough serum to exclude the non-specific results in ELISA.

Duck samples in three markets that showed positive results in ELISA were negative for AI antibody detection using the HI test; the other tested duck samples showed positive results for AI antibody detection, with a percentage ranging from 10.5 to 80. On the other hand, tested chicken samples showed positive results in ELISA for AI antibodies in all markets using the HI test, with a percentage ranging from 85.7 to 100.

The range of detection of antibodies in chickens and ducks may be due to the fact that some of the birds had been vaccinated against AI and some might not have been.

Results of detection of ND virus and ND antibodies among birds in live bird markets

Results of ND virus detection from LBMs in Tanta District in both first and second week (Table 7 and 8) showed that all examined samples were negative using real time PCR. Detection of ND antibodies using the HI test revealed the absence of antibodies against ND in duck samples in all markets except samples in one market, with a percentage of 20 percent and a geometric mean of 7. This is may be due to the fact that there is no vaccination of ducks against ND.

Regarding the detection of ND antibodies in chickens, examined samples were positive using the HI test with a percentage that ranged from 56.2 to 100, which indicates that some birds may have been vaccinated while others may be have been. The geometric mean ranged from 4.9 to 7.1, which reflects differences in the antibody level among examined birds.

Detection of ND antibodies using HI tests revealed the absence of antibodies against ND in all duck samples in all markets except in one, which showed positive results with a percentage of 4.8 and a geometric mean of 4.

Regarding the detection of ND antibodies from chickens, all samples were positive for antibody detection through HI tests, with a percentage ranging from 33.3 to 94.4, which indicates that some birds may have been vaccinated and others might not have been. The geometric mean ranged from 4.2 to 6.8, which reflected differences in the antibody level among examined birds.

Discussion

LBMs are considered a strong focus for the spread of HPAI transmission. Clearly, LBMs represent a risk for human exposure at the market or subsequently, when birds are slaughtered at home for consumption. The result of this study revealed that AI H5 virus was detected in traded poultry in four out of ten tested LBMs. Both ducks and chicken harbour the infection. The virus was detected in clinically healthy birds, but not exclusively. The virus was detected in chickens mainly from farms, whereas infected ducks were from backyards. The antibody surveillance for detection of AI antibodies indicates that not all the backyard birds receive AI vaccination. Search in viruses in the LBMs is a feasible and promising approach to determine the distribution of AI virus among poultry in Egypt and could be a useful tool to evaluate the vaccination coverage against AI vaccine. In Egypt, LBMs have the potential to spread the disease through traders that sell birds for restocking rather than for consumption. Based on the available results and observations, LBMs can be considered a potential high-risk area of spread of AI.

ND virus could not be detected in any of the collected samples either using real time PCR



or virus isolation, whereas protection of chicken against ND ranges from 33.3 to 94.4 percent, probably due to systematic ND vaccination on the farms. The status regarding ND in the households is unknown.

Due to the pooling scheme of samples, no quantitative results (prevalence) could be obtained. In future planning, the pooling should be done so as to allow the analysis of the individual samples out of the pool in the case of positive findings. It is also recommended to process the tracheal swabs separately from the cloacal swabs, as this could give an indication of the AI virus involved, with HPAI H5N1 more likely found in tracheal swabs.

In large markets (> 1 000 birds traded per market day), generally birds from farms are traded. The risk regarding the occurrence and spread of the disease is equal in small (<500 birds traded per poultry day) and large markets, but the factors are different. In large markets, the number of birds from different sources kept in a limited space favours the contamination, whereas in small markets, the diversity and the number of sources pose a threat.

Spread of AI virus could occur because villagers and sellers move birds from one market to another.

It was difficult to get accurate information from villagers and sellers, and to trace the source of poultry, since they were reluctant to share their knowledge with unfamiliar people. The survey team suggests using local people for collecting the information, for example local veterinarians, preferably women. Another approach would be to separate the information gathering exercise from the sampling.

Conclusions and recommendations

The survey done in Tanta District has shown that AI H5 virus detection in the poultry population in LBMs is feasible and valuable. It may help detect “hot spots” of H5N1 infection. It does not allow tracing back to specific sources (farms, households), but with an appropriate pooling scheme, it may be possible to attribute the positive cases to either the commercial breeds (chicken and ducks) or to the *Balady* system. The characteristics of the *Balady* system are the use of local crossbred chickens and, production of chicks mainly through traditional hatcheries and poor biosecurity.

As the marketing pattern is unknown, it was also not possible to trace the positive cases to a specific region.

It is recommended to redesign the survey before extending it to other districts. The survey team recommends first carrying out a mapping and description of the markets, as was done in Cambodia. Based on the results compiled in the database, the sampling would be planned accordingly to the size of the markets (number of samples) and to the different “poultry seasons” (spring, Ramadan, Christmas, etc.). FAO would provide the needed software and training for the mapping exercise.



Annexes

Annex 1

Table 1. Live bird markets in Tanta District, Gharbia Governorate

Serial number	Village	Day of the market	Source of traded birds	Ratio of chicken to ducks/geese
1*	Berma	Wednesday	Backyard farms	1:01
2	Hesa Shabser	Wednesday	Backyard	5:03
3	Kaniset Demshet	Thursday	Backyard farms	2:01
4*	Cohafa	Daily	Backyard farms	2:01
5	El Ragdeia	Thursday and Saturday	Backyard farms	n.a.
6	Sebrbay	Friday	Backyard	1:02
7	Shobra El Namla	Saturday	Backyard farms	2:01
8	Mahalet Marhom	Saturday	Backyard farms	2:01
9	Shony	Sunday	Backyard	1:02
10	Talbert Kayser	Sunday	Backyard farms	2:01
11	El Sebaee	Daily	Backyard farms	5:01
12	Shayki	Daily	Backyard farms	9:01
13*	EL Hekma	Monday and Friday	Backyard farms	4:01
14	El Gaban	Friday	Backyard farms	3:01
15	Nawag	Wednesday and Sunday	Backyard farms	2:01
16	Nefia	Daily	Backyard farms	3:01
17	Defra	Monday	Backyard farms	2:01
18	Mahlat Rouh	Monday	Backyard farms	4:01
19	Akhnawai	Monday and Friday	Backyard	6:01
20	Meet Hebesh El Bahr	Daily	Backyard farms	4:01
21	Sanaded	Monday and Friday	Backyard	n.a.
22*	Mahalet Monoof	Tuesday	Backyard farms	2:01
23	Kherset	Daily	Backyard farms	2:01
24	Shobar	Saturday and Tuesday	Backyard farms	3:02

* Shaded markets are the surveyed ones.

Figure 1: Ratio of chicken to ducks and geese in the selected markets

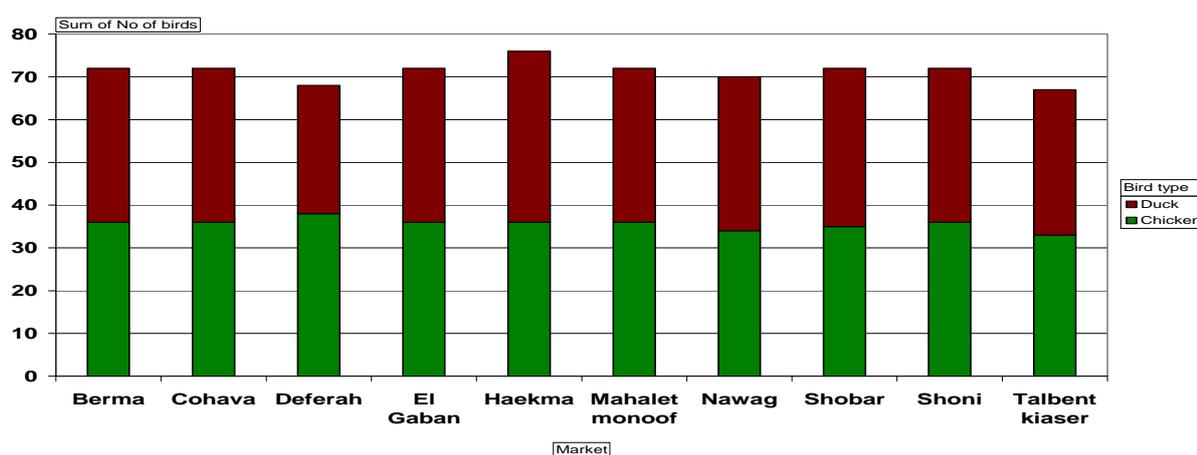


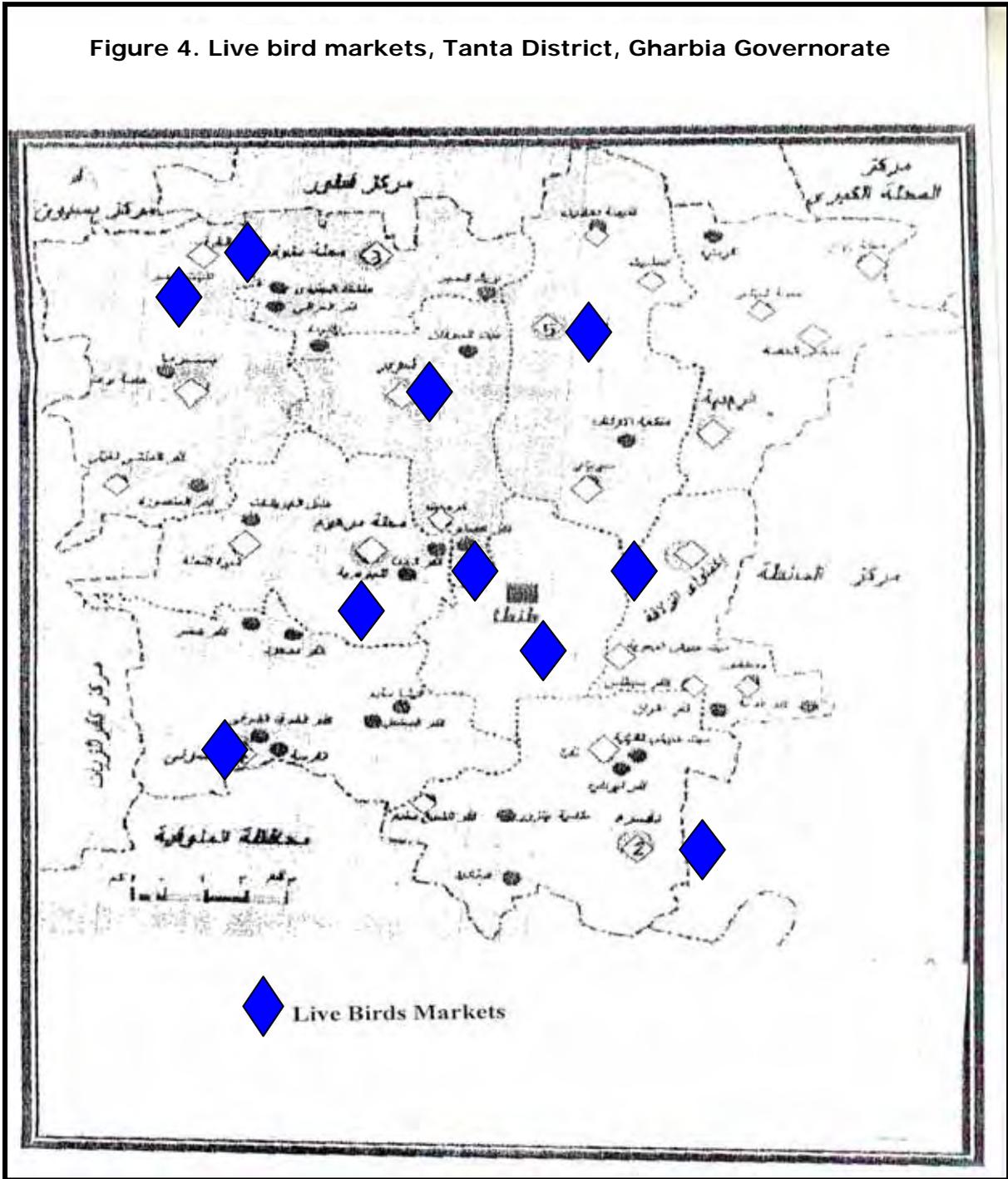
Figure 2. Map of Egypt with Gharbia Governorate



Figure 3. Gharbia Governorate and its districts



Figure 4. Live bird markets, Tanta District, Gharbia Governorate



Annex 2

General information on the live bird markets

1. Location of the market – village name, district name, governorate name, GPS coordinates
2. Is the market formal or informal?
3. What is the size of the live bird market (no. of birds)?
4. Are there other animals traded (rabbits, goats, and sheep)?
5. How often are there market-days per week?
6. How often are there birds sold? Are there special “poultry days”?
7. How many and what kind (species, age) of birds are sold – on average? - on “poultry days”?
8. Are the species separated in the market place?
9. Are the birds slaughtered on the spot?
10. Is there a specific place for the slaughtering?
11. Are there provisions in place for the disposal of carcasses and offal?
12. What happens to birds that are not sold at the end of the day?

Information from the seller

Is the seller the producer (farm/backyard) or a family member?

1. If not, are the birds from different sources (farms or backyards)?
Where are the birds from?
 - the same district
 - the same Governorate
 - other places(if a mixture of all the cases, please indicate the proportions).
2. What type of production system do the birds come from—backyard or farm, or both?
3. Does the seller also provide birds to other markets? Where?



Annex 3

Table 2. The tabulated data of the surveyed markets at Tanta district Gharbia Governorate

Market	Species	Type of bird	Date of market	Size of market	Other animals	How are the birds sold?	Where do the birds come from?	Source	The seller sells in another market?	Formality
Shony	Chicken -duck	Broiler	Sunday	Small: <500 birds	Rabbit	Live for home slaughter	Same district	Backyard-farms	Yes	Informal
Talbent Kayser	Chicken -duck	Breed	Sunday	Small: <500 birds	Rabbit	Live for home rearing	all districts in the governorates	Backyard-farms	Yes	Informal
Defra	Chicken -duck	Broiler	Monday	Small: <500 birds	Rabbit Sheep Goat	Live for home slaughter or rearing	all districts in the governorates	Backyard-farms	Yes	Informal
Mahalet Monoof	Chicken -duck	Broiler-layer-Breed	Tuesday	Small: <500 birds	None	Live for home slaughter or rearing	Berna	Backyard-farms	Yes	Informal
Shobar	Chicken -duck	Broiler-layer-	Tuesday	Small: <500 birds	Rabbit	Live for home slaughter or rearing	Same district	Backyard-farms	Yes	Informal
Nawag	Chicken -duck	Broiler-layer-Breed	Wednesday	Small: <500 birds	Rabbit	Live for home slaughter or rearing	Same district	Backyard-farms	Yes	Informal
Berma	Chicken -duck	Broiler-layer-Breed	Wednesday	Large: >1000 birds	Rabbit	Live for home slaughter or rearing	all districts in the governorates	Backyard-farms	Yes	Informal
El Hekma	Chicken -duck	Broiler-layer-Breed	Monday -Friday	Large: >1000 birds	Rabbit	Live for home slaughter or rearing	all districts in the governorates	Backyard-farms	Yes	Informal
El Gaban	Chicken -duck	Broiler-layer-Breed	Thurs-Monday	Mid-size: 500-1000 birds	Rabbit	Live for home slaughter or rearing	all districts in the governorates	Backyard	Yes	Informal
Cohafa	Chicken -duck	Broiler-Breed	Thurs-Monday	Mid-size: 500-1000 birds	Rabbit	Live for home slaughter	all districts in the governorates	Backyard-farms	Yes	Informal

Markets with positive AI-V findings in Week 1

Market with positive AI-V findings in Weeks 1 and 2



Table 3. Results of AI detection in live bird markets surveillance using RRT-PCR and isolation in Tanta District, Gharbia Governorate, 16–27 March 2008 (Week 1)

Code	Market	Bird type	Vaccination	RRT-PCR AI Result			AI isolation
				Common	H5	H7	
G1	Shony	Duck	UC**	Positive	Positive	Negative	Positive
		Chicken	UC	Positive	Negative	Negative	Positive
G2	Talabent kaysar	Duck	UC	Positive	Positive	Negative	Positive
		Chicken	UC	Positive	Positive	Negative	NI*
G3	El Hekma	Duck	Not vaccinated	Positive	Positive	Negative	Positive
		Chicken	7 out of 18	Positive	Positive	Negative	Positive
G4	Defra	Duck	Vaccinated	Negative	NT***	NT	Negative
		Chicken	11 out of 19	Negative	NT	NT	Negative
G5	Mahalt monoof	Duck	Not vaccinated	Negative	NT	NT	Negative
		Chicken	Not vaccinated	Negative	NT	NT	Negative
G6	Shobar	Duck	13 out of 15	Negative	NT	NT	Negative
		Chicken	UC	Negative	NT	NT	Negative
G7	Nawag	Duck	UC	Negative	NT	NT	Negative
		Chicken	UC	Negative	NT	NT	Negative
G8	Perma	Duck	UC	Negative	NT	NT	Negative
		Chicken	UC	Negative	NT	NT	Negative
G9	El gaban	Duck	Not vaccinated	Positive	Positive	Negative	Positive
		Chicken	Not vaccinated	Negative	NT	NT	Negative
G10	Cohafa	Duck	2 out of 10	Negative	NT	NT	Negative
		Chicken	UC	Negative	NT	NT	Negative

*NI: not isolated after two blind passages in SPF ECE

**UC = Unknown condition

***NT= Not tested



Table 4. Results of AI detection of LBM surveillance using RRT-PCR and isolation, Tanta District, El-Gharbia Governorate, 16–27 March 2008 (Week 2)

Code	Market	Bird type	Vaccination	RRT-PCR AI Result			AI isolation
				Common	H5	H7	
G11	Talabent kaysar	Duck	UC**	Negative	NT***	NT	Negative
		Chicken	UC	Positive	Positive	Negative	NI*
G12	Shony	Duck	UC	Negative	NT	NT	Negative
		Chicken	UC	Negative	NT	NT	Negative
G13	El Hekma	Duck	UC	Negative	NT	NT	Negative
		Chicken	UC	Negative	NT	NT	Negative
G14	Defra	Duck	4 out of 19 vaccinated	Negative	NT	NT	Negative
		Chicken	UC	Negative	NT	NT	Negative
G15	Shobar	Duck	UC	Negative	NT	NT	Negative
		Chicken	UC	Negative	NT	NT	Negative
G16	Mahalet Monoof	Duck	UC	Negative	NT	NT	Negative
		Chicken	Vaccinated	Negative	NT	NT	Negative
G17	Perma	Duck	UC	Negative	NT	NT	Negative
		Chicken	UC	Negative	NT	NT	Negative
G18	Nawag	Duck	Not vaccinated	Negative	NT	NT	Negative
		Chicken	17 out of 18	Negative	NT	NT	Negative
G19	Cohafa	Duck	Not vaccinated	Negative	NT	NT	Negative
		Chicken	14 out of 18 vaccinated	Negative	NT	NT	Negative
G20	El gaban	Duck	UC	Negative	NT	NT	Negative
		Chicken	UC	Negative	NT	NT	Negative

*NI: not isolated after two blind passages in SPF ECE

**UC = Unknown condition

***NT = Not tested



Table 5. Results of AI detection of LPM surveillance using ELISA and

Code	Market	Vaccination	AI antibody detection by ELISA test		AI antibody detection by HI test	
			Positive/total	Percentage ¹	Percentage ²	Positive HI / positive ELISA
G1	Shony	UC*	7/18	38.9	85.7	6/7
		UC	10/16	62.5	88.9	8/9
G2	Talabent kaysar	UC	11/17	64.7	87.5	7/8
		UC	13/18	72.2	100	13/13
G3	El Hekma	Not vaccinated	12/16	75	63.6	7/11
		7 out of 18 vaccinated	10/13	76.9	88.9	8/9
G4	Defra	Vaccinated	7/11	63.6	66.7	4/6
		11 out of 19 vaccinated	9/15	60	100	5/5
G5	Mahalet monoof	Not vaccinated	5/17	29.4	20	1/5
		Not vaccinated	11/16	68.7	85.7	6/7
G6	Shobar	13 out of 15 vaccinated	5/5	100%	50	2/4
		UC	15/18	83.3	90.9	10/11
G7	Nawag	UC	6/16	37.5	100	5/5
		UC	6/17	35.3	100	6/6
G8	Perma	UC	13/18	72.2	100	13/13
		UC	7/18	38.9	100	7/7
G9	El gaban	Not vaccinated	4/14	28.6	100	3/3
		Not vaccinated	9/17	52.9	87.5	7/8
G10	Cohafa	2 out of 10 vaccinated	5/10	50	100	5/5
		UC	12/18	66.7	100%	12/12

HI tests, Tanta District El-Gharbia Governorate; 16-27 March 2008 (Week1)

*UC = Unknown condition

¹ The percentage refers to the number of positive samples out of the total number of samples of the same market.

² The percentage refers to the number of positive HI findings. Only when enough material was available, an HI test was done to confirm the ELISA test results.



Table 6. Results of AI antibody detection in live poultry market (LPM) surveillance using ELISA and HI tests, Tanta District, El-Gharbia Governorate; 16-27 March 2008 (Week 2)

Code	Market	Birds	Vaccination	AI antibody detection through the ELISA test		AI antibody detection through the HI test	
				Percentage	Positive/ total	Percentage	Positive HI / ELISA test
G11	Talabent kaysar	Duck	*UC	33.3	5/15	80	4/5
		Chicken	UC	29.4	5/17	100	4/4
G12	Shony	Duck	UC	29.4	5/17	80	4/5
		Chicken	UC	57	8/14	85.7	6/7
G13	El Hekma	Duck	UC	27.8	5/18	0	0/5
		Chicken	UC	53	9/17	100	9/9
G14	Defra	Duck	4 out of 19 vaccinated	36.8	7/19	71.4	5/7
		Chicken	UC	58	9/17	100	10/10
G15	Shobar	Duck	UC	23.8	5/21	0	0/5
		Chicken	UC	75	12/16	100	11/11
G16	Mahalet Monoof	Duck	UC	27.8	5/18	13	3/23
		Chicken	Vaccinated	81	13/16	100	11/11
G17	Perma	Duck	UC	0	0	29.4	5/17
		Chicken	UC	61	11/18	100	11/11
G18	Nawag	Duck	Non-vaccinated	0	0	10.5	2/19
		Chicken	17 out of 18	0	0	0	0/0
G19	Cohaafa	Duck	Not.vaccinated	0	0	0	0/0
		Chicken	14 out of 18	50	9/18	100	9/9
G20	El gaban	Duck	UC	43.7	7/16	100	9/9
		Chicken	UC	22.2	4/18	100	4/4

*UC = Unknown condition



Table 7. Results of ND detection in live poultry markets (LPMs) surveillance using RRT and HI tests, Tanta District, El-Gharbia, Governorate, 16–27 March 2008 (Week1)

Code	Market	Village	Bird type	RRT-PCR ND Result	Antibody detection for ND through the HI test
					Percentage
G1	Shony	Gharbia Tanta Shony	Duck	Negative	0
			Chicken	Negative	56.2
G2	Talabent kaysar	Gharbia Tanta, T. kaysar	Duck	Negative	0
			Chicken	Negative	88.8
G3	Al-Hekma	Gharbia Tanta	Duck	Negative	0
			Chicken	Negative	92.3
G4	Defra	Gharbia, Tanta, Dafra	Duck	Negative	20
			Chicken	Negative	73.3
G5	Mahalet monoof	Gharbia Tanta	Duck	Negative	0
			Chicken	Negative	81.2
G6	Shobar	Gharbia Tanta, Shobar	Duck	Negative	0
			Chicken	Negative	94.4
G7	Nawag	Gharbia Tanta	Duck	Negative	0
			Chicken	Negative	76.5
G8	Perma	Gharbia	Duck	Negative	0
		Tanta	Chicken	Negative	94.1
G9	El Gaban	Gharbia	Duck	Negative	0
		Tanta	Chicken	Negative	100
G10	Cohafa	Gharbia Tanta	Duck	Negative	0
			Chicken	Negative	94.4



Table 8. Results of ND detection surveillance in LPM using RRT-PCR and HI tests, Tanta District, El-Gharbia Governorate, 16-27 March 2008 (Week 2)

Code	Market	Bird type	RRT-PCR ND Results	Antibody detection for ND using the HI test
				Percentage
G11	Talabent kaysar	Duck	Negative	Negative
		Chicken	Negative	68
G12	Shony	Duck	Negative	Negative
		Chicken	Negative	38.4
G13	El Hekma	Duck	Negative	Negative
		Chicken	Negative	94.1
G14	Defra	Duck	Negative	Negative
		Chicken	Negative	89.5
G15	Shobar	Duck	Negative	Negative
		Chicken	Negative	93
G16	Mahalet Manoof	Duck	Negative	Negative
		Chicken	Negative	80
G17	Perma	Duck	Negative	Negative
		Chicken	Negative	94.4
G18	Nawag	Duck	Negative	Negative
		Chicken	Negative	76.5
G19	Cohaafa	Duck	Negative	Negative
		Chicken	Negative	66.6
G20	El Gaban	Duck	Negative	4.8
		Chicken	Negative	33.3

Table 9. No. of samples and their sources, and no. of pooled samples (Week 1)

Market	Size	No. of sample	No. of pools		No. of positive pools		Sample source				Source of positive sample				
			Ch	Du	Ch	Du	Ch		Du		Chicken		Du		
			Farm	BY	Mix	BY	Farm	BY	Mix	BY					
SHONY	Small	17	18	4	4	1	3	-	-	4	4	-	-	1	3
TALBENT KAYSER	Small	18	17	4	4	2	1	4	-	-	4	2	-	-	1
EI HEKMA	Large	18	18	4	4	1	1	1	-	3	4	1	-	-	1
DEFRA	Small	17	11	4	2	-	-	4	-	-	2	-	-	-	-
MAHALET MONOOF	Small	15	13	4	3	-	-	4	-	-	3	-	-	-	-
SHOBAR	Small	18	14	4	3	-	-	4	-	-	3	-	-	-	-
NAWAG	Small	18	17	4	4	-	-	3	-	1	4	-	-	-	-
BERMA	Large	18	17	4	4	-	-	-	3	1	4	-	-	-	-
EL GABAN	Mid-Size	18	14	4	3	-	3	2	1	1	3	-	-	-	3
COHAFA	Mid-Size	18	10	4	2	-	-	3	-	1	2	-	-	-	-
TOTAL		175	149	40	33	4	8	25	4	11	33	3		2	5

*Ch=Chicken, Du=Duck



Table 10: Number of samples and their sources and number of pooled samples (week 2)

Market	Size	No. of samples		No. of pools		No. of positive pools		Sample source						Source of AI positive samples through the RT-PCR			
		Duck	Chicken	Duck	Chicken	Duck	Chicken	Duck	Chicken			Duck			Chicken		
									Farm	Backyard	MIX	Farm	Backyard	MIX	Farm	Backyard	MIX
SHONY	Small	18	18	4	4	-	-	2	-	2	-	4	-	-	-	-	-
TALBENT KAYSER	Small	19	19	4	4	1	-	3	-	1	-	4	-	-	-	-	1
EL HEKMA	Large	16	22	4	4	-	-	2	2	-	1	3	-	-	-	-	-
DEFRA	Small	18	19	4	3	-	-	4	-	-	-	4	-	-	-	-	-
MAHALET MONOOF	Small	18	26	4	4	-	-	1	3	-	-	3	1	-	-	-	-
SHOBAR	Small	18	22	4	4	-	-	3	1	-	-	4	-	-	-	-	-
NAWAG	Small	18	18	4	4	-	-	3	-	1	-	4	-	-	-	-	-
BERMA	Large	18	16	4	4	-	-	4	-	-	-	4	-	-	-	-	-
EL-GABAN	Mid-size	16	22	4	4	-	-	3	-	1	-	4	-	-	-	-	-
COHAFA	Mid-size	19	18	4	3	-	-	4	-	-	-	4	-	-	-	-	-
TOTAL		178	200	40	38	1	0	29	6	5	1	38	1		-	1	



