

Field evaluation of common poultry viral vaccines in Egypt: a need for reassessment of the vaccine value chain

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Newcastle disease,
Infectious bronchitis.

Summary

Egypt has a large traditional and exotic poultry sector which is challenged regularly by poultry diseases in endemic and epidemic proportions. The household poultry in particular is a source of livelihoods and employment for millions of low income citizens. Highly pathogenic avian influenza (HPAI) H5N1 and Newcastle disease are the most important poultry diseases in this sector. Whereas poultry vaccines are available to reduce the incidence of disease in Egypt, their effectiveness is doubtful. We conducted a biological evaluation of selected viral vaccines of poultry in three governorates in Egypt. Fifty-four percent of the vaccines had reduced vaccine titres and the effect of secondary vaccine distributions was associated with the observed vaccine titres. External contamination was observed in some vaccines and break in cold chain was reported. Whereas no vaccine distributor used purpose-built vaccine refrigerator, none also had prescribed protocol for vaccine handling or kept record of vaccine. There is a need to review vaccine handling procedure, monitor of vaccine cold chain more critically and review the whole chain that support vaccine distributions in Egypt.

Controllo sulla gestione, manutenzione e distribuzione dei vaccini aviari in Egitto

Parole chiave

Vaccino,
Catena del freddo,
Valutazione del vaccino,
Egitto,
Influenza aviaria
Malattia di Newcastle,
Bronchite infettiva.

Riassunto

L'industria avicola egiziana è fonte di sostentamento e di occupazione per milioni di cittadini a basso reddito. L'avicoltura, tuttavia, è un settore sovente coinvolto in episodi di malattie endemiche ed epidemiche. Tra queste, l'influenza aviaria ad alta patogenicità e la pseudopeste sono sicuramente quelle più importanti. Sebbene in Egitto siano disponibili e utilizzati vaccini in grado di ridurre l'incidenza di tali malattie, resta dubbia la loro efficacia. In questo studio sono stati valutati alcuni vaccini virali utilizzati per gli allevamenti avicoli in tre governatorati egiziani. Nel 54% dei prodotti selezionati è stata riscontrata una diminuzione del titolo virale spesso correlata a problemi riscontrati nella catena di distribuzione dei vaccini, come presenza di contaminanti sui flaconi vaccinali o interruzione della catena del freddo. I frigoriferi dove sono conservati i prodotti non sono appropriati per la conservazione e stoccaggio di vaccini né sono presenti procedure per la loro manutenzione e stoccaggio. È rivedere o stilare nuove procedure di gestione, manutenzione e distribuzione dei vaccini che prevedano un maggior controllo e rispetto della catena del freddo.

Introduction

Egypt has a large poultry sector with more than 50 thousands commercial producers and the Egyptian poultry farming systems include the exotic broilers and layer chicken, indigenous (Baladi) chicken, ducks (Peking, Muscovy, Mule and Sudani breeds), turkey, geese, ostriches, and quails. Day old birds are supplied by both, the traditional and modern hatcheries. At any one time, the total standing poultry population is approximately one billion birds with the commercial poultry sector contributing approximately 90% while the remaining 10% originate from the small-scale holders and household poultry. These poultry farms are particularly spread in villages and near cities in Egypt (Ali *et al.* 2013).

The household poultry production is a source of livelihoods and employment for millions of low income citizens. The sector is marked with complex marketing chains and diverse challenges including diseases of considerable economic and public health significance. In the last decade, the financial losses caused by the major epidemic diseases of poultry such as highly pathogenic avian influenza (HPAI) H5N1 and Newcastle disease (ND) have been enormous (Marangon and Busani 2007, Fasina *et al.* 2008).

Vaccines are widely used in the prevention and reduction of incidence as well as control of endemic poultry diseases. For a vaccine to be useful in the maintenance of animal health, it must be pure, safe, potent, and effective (OIE 2014). In Egypt, approximately 10.4 billion doses of vaccines were used in 2014, the majority (96.43%) of which were imported while only 3.57% were produced locally (Central Laboratory for Evaluation of Veterinary Biologics - CLEVB, unpublished data). Of these doses, poultry vaccines accounted for 99.7% with an estimated end user value of \$236 million (1.7 billion Egyptian Pounds, CLEVB unpublished data).

In Egypt, the CLEVB is the authorized governmental laboratory for the evaluation and certification of veterinary vaccines and biologicals prior to their release into the markets. The organisation complies with best standards and it is an ISO/EIC 17025 accredited laboratory which handles both imported and locally produced vaccines. Multi-level vaccine distributions are done primarily using refrigerated trucks to more than 5,000 district and village stores, and further downstream supplies reach the over 50,000 commercial poultry farms. Whereas, cold rooms are available in the large integrated farms and with few big distributors, vaccines are kept in the kitchen-type and household model refrigerators from where the numerous household producers only collect them in small quantities as ready-to-use products.

While the recommended cold chain temperature for vaccine maintenance globally is + 2 °C to + 8 °C, the Marek's disease vaccine and many of the recombinant vaccines should be kept in liquid nitrogen (- 196 °C) (Techathawat *et al.* 2007, Kumru *et al.* 2014). From these vaccine storage facilities, supplies are distributed in smaller cold boxes or vaccine carriers to end users. Although the success of vaccination programmes in poultry depends partially on the quality of manufactured vaccines, it also depends on handling, the maintenance of cold chain along the marketing and transport processes and the correct application of vaccines to the recipient birds (Collett 2013). In addition, the shelf life, potency and immunogenicity of vaccines will determine the level of immunity that an animal develops following vaccination¹.

For a veterinary vaccine to maintain its potency and immunogenicity, it must not only be stored at the required temperature of + 2 °C to + 8 °C, it must also be maintained in a "cold chain" – a continuum of safe handling and procedures on the vaccine from the time of manufacture through the transport processes until used in animals (Subramanyam 1989, Cheyne 1989, Chojnacky *et al.* 2010). Previous reports have confirmed that incorrect storage temperature (Miller and Harris 1994, Nelson *et al.* 2004), interference with maternally derived antibodies (Kim *et al.* 2010), inadvertent placement of vaccines outside the refrigerator and certain other practices like incorrect dilutions and unprofessional administration have all affected immunogenicity and responses to vaccines (Miller and Loomis 1985, Sockey *et al.* 1988, Casto and Brunell 1991).

Despite the intense vaccination of poultry particularly against H5N1 HPAI and ND among others, anecdotal evidences from animal health practitioners and farmers, including the continuing and on-going outbreaks of avian influenza H5N1 and ND have suggested gross vaccine failures and previous scientific evaluations have confirmed these reports (Kim *et al.* 2010, Arafa *et al.* 2012, Kilany *et al.* 2015). In this study, we randomly selected viral vaccines of poultry in three poultry-dense governorates in Egypt, conducted field and laboratory evaluations in order to determine whether the value chain (from handling, cold chain practices, marketing, delivery and use at the farm levels) affects potency, viability, field efficacy and immunogenicity with consequent effect on efficacy.

¹ Poultry Hub. 2015. Vaccination by Poultry CRC, 2015. <http://www.poultryhub.org/health/health-management/vaccination/> accessed 2 August 2015.

Materials and methods

Study areas and participants' recruitment

Three governorates (Sharqia, Dakahlia and Fayoum) were purposively selected based on their relative importance as poultry-dense locations in Egypt. In each governorate, two geographically distinct regions were selected, including a more central location, usually in the capital of each governorate where most of the primary vaccine distributors were based, and secondly, a distant district which had high densities of poultry populations with large numbers of secondary distributors. All district-level vaccine distributors were identified in close consultation with the Governorates' Veterinary Services. The vaccine distributors were classified into: primary (main) or secondary (sub-) distributors based on their level of involvement in vaccine distribution. While the primary distributors get poultry vaccines directly from the manufacturing companies or their agencies in Egypt and sell them in bulk, the secondary distributors serve as links between the primary distributors and poultry farmers. All participants in the study willingly participated and gave consent for the work to be published. They were informed that they have the right to discontinue participation at any time in the course of the study.

Vaccine sample collection and analysis

Eight primary distributors (2 from Sharqia, 3 from Dakahlia and 3 from Fayoum) and 15 secondary distributors (5, 4, and 6 from Sharqia, Dakahlia and Fayoum, respectively) were selected. A total of 41 viral vaccines of poultry were collected and recruited for the study, including 30 live vaccines and 11 inactivated/killed vaccines. Of this total, 33 were monovalent ($n = 33$) vaccines and 4 were bivalent ($n = 8$) vaccines, and these consist of 24 ND, 6 infectious bursal disease (IBD), 4 infectious bronchitis (IB), 5 avian influenza, 1 pox and 1 avian encephalomyelitis (AE) vaccines. These vaccines originated from the 23 distribution points mentioned above. For a vaccine to qualify for inclusion in the study, it had to fulfil the following criteria: (a) it had to be collected from any of the 23 identified distributors within Sharqia, Dakahlia or Fayoum, (b) it had to have been previously evaluated and had to have reserve batch samples kept at CLEVB to enable paired comparison, and (c) it had to be collected and transported immediately to the laboratory in a temperature-monitored transport box whose temperature was maintained consistently between + 2 °C to + 8 °C. Vaccine from any box with temperatures outside this range was not included in the study.

At the same time that the vaccines were collected, cold chain storage facilities at the distribution points were inspected and assessed using a validated questionnaire and a checklist (Table I). The checklist was a modified guide from the Centers for Disease Control and Prevention, USA (CDC 2003) and the US Army Medical Material Agency document (USAMMA 2005). Temperature readings were taken from the refrigerators using calibrated thermometers placed in the middle of the storage area (refrigerator) for at least 20 minutes while the questionnaire was answered (Bell *et al.* 2001).

At each sampling location, preference was given to collection of the oldest batch where available, and/or those with apparent physical defacement or abnormality. Collected vaccines were labelled and transported directly to CLEVB in ice boxes with hourly temperature taken during transportation with the aid of computerized transit temperature data loggers (range: - 40 °C to + 85 °C). Any box with logged temperature data outside the range of + 2 °C to + 8 °C were excluded and all accepted vaccine vials were stored at CLEVB facility until evaluated. The median time for vaccine transport from all distributors to the CLEVB was 4 hours 18 minutes.

Questionnaires

A twenty-two item pre-tested questionnaire was administered to each of the selected vaccine distributors ($n = 23$, Table I) to collect information on the procedures and processes around vaccine handling and management of vaccine refrigerators. The instrument was prepared in English and Arabic language and all answers were recorded in a Microsoft Excel® spreadsheet. Checklist was used to assess the consistency of the answers against practices at the vaccine distribution stores and where significant deviations from the answers given were observed, a reconfirmation was obtained or the details from the checklist based on observation was utilised.

Assay procedures

The vaccines retrieved from the distributors and their corresponding reserved batches previously retained at CLEVB were subjected to parallel evaluations by virus titration (for live vaccines) and serological immune response (for inactivated vaccines) according to standard protocols (OIE 2014).

Virus titration for live (attenuated) poultry vaccine

Newcastle disease (ND)

Ten-fold serial dilutions (10^{-1} to 10^{-9}) of ND, IBD and IB

Table 1. Factors affecting vaccine immunogenicity, potency and titre in 23 veterinary vaccines storage points in Egypt.

Variables with effect on vaccine efficacy and immunogenicity	Yes	%	No	%	P-value
Cold chain system has a purpose-built refrigerator (domestic/household refrigerators are not suitable for storage of vaccines).	0	0	23	100	NA
The refrigerator is situated away from direct heat source.	13	56	10	44	0.77
Refrigerator plug is protected e.g. encased to prevent tampering; security marked "Do Not Switch Off" or is hardwired ('spurred').	0	0	23	100	NA
Refrigerator is cleaned and defrosted if necessary and regularly (at least every 6 months)	18	78	5	22	0.0001
Only pharmaceutical items and biological are stored in the refrigerator i.e. no food, drink are stored in the refrigerator.	10	44	13	56	0.38
A thermometer is available to monitor temperature routinely.	5	22	18	78	0.0001
The thermometer can give the minimum, current and maximum readings daily.	0	0	23	100	NA
Procedures are in place for at least daily recording of temperatures.	0	0	23	100	NA
Recording form or equivalent is used.	0	0	23	100	NA
Minimum, maximum and actual temperature is checked and recorded.	0	0	23	100	NA
Procedures are in place for action to be taken in the event of abnormal temperatures.	0	0	23	100	NA
Vaccines are stored in the cabinet of the refrigerator and not in the refrigerator doors.	4	17	19	83	< 0.0001
Items are stored away from the back and sides of the refrigerator and the freezer compartment if it has one.	3	13	20	87	< 0.0001
Vaccines are not being stored in the bottom drawer of the floor of refrigerator. Unless it is a pharmaceutical refrigerator with custom made wire baskets. The 'salad' boxes of domestic refrigerators are not be used.	3	13	20	87	< 0.0001
No more than 66% of the internal volume of refrigerator is filled.	7	30	16	70	0.04
Expiry dates are checked regularly at least once a month and these records are documented and filed away.	20	87	3	13	< 0.0001
Stock rotation is carried out to ensure that the shortest expiry dates are used first.	20	87	3	13	< 0.0001
A responsibility is attached to a named person and a deputy to monitoring the refrigerator routinely.	12	52	11	48	0.77
Electricity disruptions have been experienced.	22	96	1	4	< 0.0001
These electricity outages do occur periodically.	18	78	5	55	0.0001
The vaccine store has an alternative back-up system for power outages.	11	48	12	52	0.77
Optimum refrigerator temperature kept between 2 °C and 8 °C	9	39	14	61	0.04

vaccines were prepared. Five (5) specific pathogen free (SPF) chicken embryos (9-11 days old) were inoculated with 0.2 ml of each suspension via the allantoic cavity using /egg. Five un-inoculated chicken embryos of the same age and source were kept as negative un-inoculated controls and five others were inoculated with the diluent as negative inoculated control. All embryos were incubated at 37 °C for 5-8 days and observed daily by candling. Nonspecific deaths during the first 24-hours post-inoculation were discarded. Dead embryos and those that survived during the period of observation were examined for specific lesions associated with each virus. The median egg infectious dose (EID₅₀) was calculated individually according to the method described by Reed and Muench (Reed and Muench 1938).

Avian encephalomyelitis (AE)

Tenfold serial dilution (10⁻¹ to 10⁻⁶) of the AE vaccine was prepared. Ten SPF chicken embryos (5-6 days old) were inoculated through the yolk sac with 0.2 ml of each dilution. Twenty embryos from the same source and age were kept un-inoculated as

negative controls and another set was inoculated with the diluent as negative inoculated control. All eggs inoculated with the same dilution were kept in separated tray, incubated at 37 °C and candled every 24 hours. Dead embryos during the first 48 hours post inoculation were discarded as non-specific. All embryonated eggs were allowed to hatch and the chicks were placed in a brooder and monitored for clinical manifestation of AE for 3 days.

The number of chicks that manifested clinical signs of AE were identified and recorded. The EID₅₀ was calculated as previously mentioned (Reed and Muench 1938).

Fowl pox vaccine (FPV)

Tenfold serial dilution (10⁻¹ to 10⁻⁹) of the FPV was prepared. Five SPF chicken embryos (10-12 day old) were inoculated with 0.2 ml from each dilution on the chorio-allantoic membranes (CAMs). Five days post inoculation, the surviving embryos were examined for evidence of pock lesions. CAMs with pock lesions were enumerated as positive and EID₅₀ was calculated as described above.

Tissue culture vaccine (IBD live vaccines)

Tenfold serial dilution (10^{-1} to 10^{-8}) of the IBD was prepared in a tissue culture micro-titer plate containing confluent monolayers of chicken embryo fibroblast cells. Five wells were inoculated with each dilution using 100 μ l/well. Five wells were inoculated with positive controls according to the viral vaccine used and five wells were left un-inoculated as negative control. The plate was sealed, incubated at 37 °C in 5% CO₂ atmosphere for 5-6 days and examined daily for evidence of cytopathic effect (CPE). Wells with CPE were counted and virus titre (TCID₅₀) was calculated (Hierholzer and Killing 1996).

Inactivated vaccine

Groups of twenty SPF chicken (aged 3-4 weeks) were vaccinated with different inactivated vaccine samples collected from the field and the retained samples at CLEVB. Each group was kept in separated isolators and were monitored for 4 weeks. Blood samples were collected on day 28 post-vaccination, sera were harvested and post-vaccination antibody responses were measured using haemagglutination-inhibition (HI) test and homologous antigens as described by Thayer and Beard (Thayer and Beard 1998).

External contamination (viral) of vaccine vials

Swabs from the external walls of the thirty live vaccines vials were collected and pooled into 3 pools. Each pooled swab was inoculated into 5 SPF eggs (9-11 days old). After 24 hours, all dead eggs were examined for the presence of haemagglutinating agents and specific lesion of IB or IBD. Allantoic fluids of survivor eggs were inoculated into SPF eggs and subjected to further examination as described above.

Results

Descriptive statistics

A total of 14 (61%) of the evaluated refrigerators in the primary and secondary distributor outlets did not maintain the optimum temperature (2 °C-8 °C) for vaccine storage and all of the refrigerators were domestic (household or kitchen model) type and not purpose-built vaccine refrigerators. The majority (56%) of the refrigerators were situated away from direct heat sources but none of these devices was protected from electrical surge. Fifty-six percent of the refrigerators had food and water for human consumption in the vaccine storage cabinets and only 22% had ordinary mercury thermometers (and not minimum-maximum thermometers) for temperature monitoring. None of the respondents recorded temperature daily,

had designed record form or actually checked daily temperature fluctuations (Table I). 30% did not fill the refrigerator to more than 66% of its volume, while 87% checked expiry dates and conducted stock rotations routinely. In 96% of the refrigerators tested, electricity interruptions were experienced and 78% had repeated interruptions periodically. Finally, only 48% had an alternative back-up system for electricity interruptions (Table I).

Forty-one vaccines in 37 vials from 23 distributors and three governorates were assessed in parallel (pairs) in the study including 30 (73%) live and 11 (27%) inactivated vaccines. Thirty-three (80%) of the vaccines were monovalent vaccines and the remaining 20% were bivalent vaccines. Twenty-six (63%) of the vaccines came from 15 secondary distributors while the remaining 15 (37%) were from eight primary distributors. By numbers of sample tested, ND vaccines were 24 (59%), IBD (15%), avian influenza (12%), IB (10%) and 2% each for pox virus and the AE vaccines. Some 15 (37%) of the vaccines came from Sharqia, while 31.5% each came from Dakahlia and Fayoum (Table II and III).

Vaccine titres

Reduced vaccine HI titres was observed in 20 (54%) of the 37 vials compared with their retained batch at CLEVB representing a total of 21 individual vaccines. Of the 24 tested ND vaccine samples, eleven (45.8%) showed slight to marked decrease in vaccine titres while 66.7% of the six IBD vaccines failed to maintain titres compared with the retained batch at CLEVB (Table II and III). None of the avian influenza vaccine samples maintained vaccinal or HI titres ($n = 5$) and none of the IB vaccines ($n = 4$) had reduced vaccinal titres. The only pox vaccine had failed with reduced titre but the AE vaccines maintained its vaccinal titre (Table II and III).

By volume from distribution sources and titres, no significant difference was observed between the reduced titres from primary distributors (53.3%) and secondary producers (50.0%) ($P = 0.84$). Seven of the 15 vaccines (46.7%) from Sharqia, five of the 13 vaccines from Fayoum and 9 of the vaccines from Dakahlia had reduced vaccinal or HI titres (Table II and III). There was no specific pattern in vaccinal titre reduction with regards to vaccine producers since vaccines originating from eighteen out of nineteen individual producers had reduced vaccinal or HI titres. It would appear that the secondary distributors had some positive influence on the vaccinal or HI titres of the vaccines (Table IV).

External contamination (virus) of vaccine vials

Two out of the three pools showed no embryo

lesions or produced haemagglutination when compared with the controls. The third pool showed slight haemagglutination activities with chicken red blood cells. The haemagglutinating agent was tested against specific ND H5N1 and H9N2 antisera. The contaminant was positive only against ND antiserum with a mean death time (MDT) of 98 hours.

Discussions

Vaccine remains an important component of poultry disease prevention and control measures

in Egypt and elsewhere. Their use in poultry production is aimed at avoiding or minimizing the emergence of clinical disease at farm level and to increase production. We evaluated forty-one poultry vaccines from the field in Egypt and established inconsistencies in the level of vaccinal or HI titres as a measure of immunogenicity in 54% of these vaccines. While the observation cannot be linked directly to vaccine producers due to lack of more definitive empirical data, the same cannot be said of the distribution points. A number of the interviewed distributors confirmed that although they did not assess their vaccines for quality and

Table II. Comparison of live poultry field vaccine samples with batch samples retained at CLEVB.

S/no.	Vaccine	Source	Governorate	Tested virus	Log titer of field sample* (EID ₅₀ /dose)	Log titer of CLEVB Sample (EID ₅₀ /dose)	Storage temperature of field samples (°C)
1	ND LaSota	SD	Sharqia	NDV	≥ 7.5	≥ 7.5	15.0
2	ND La Sota	PD	Sharqia	NDV	≥ 7.5	≥ 7.5	7.5
3	IBD	SD	Sharqia	IBD	5.3	5.3	10.4
4	ND LaSota	PD	Sharqia	NDV	6.5	7.1	6.5
5	IB	PD	Fayoum	IBV	≥ 5.5	≥ 5.5	4.5
6	ND LaSota,	PD	Fayoum	NDV	≥ 7.5	≥ 7.5	10.0
7	ND LaSota	SD	Fayoum	NDV	≥ 7.5	≥ 7.5	3.8
8	ND Clone	PD	Fayoum	NDV	6.9	≥ 7.5	10.5
9	ND HitchnerB1	SD	Sharqia	NDV	≥ 7.5	≥ 7.5	11.5
10	ND LaSota	SD	Sharqia	NDV	≥ 7.5	≥ 7.5	11.5
11	IBD	SD	Sharqia	IBD	5.1	5.3	11.5
12	IBD	SD	Sharqia	IBD	4.4	≥ 4.8	5.3
13	ND LaSota	SD	Sharqia	NDV	≥ 7.5	≥ 7.5	8.2
14	IBD	SD	Fayoum	IBD	≥ 4.9	≥ 4.9	9.5
15	ND LaSota	SD	Fayoum	NDV	≥ 7.5	≥ 7.5	8.9
16	IB	SD	Fayoum	IBV	≥ 5.5	≥ 5.5	7.0
17	ND HitchnerB1	SD	Fayoum	NDV	7.1	≥ 7.5	5.5
18	ND LaSota	PD	Dakahlia	NDV	7.1	≥ 7.5	12.1
19	ND LaSota	SD	Dakahlia	NDV	7.1	≥ 7.5	11.5
20	IBD	SD	Dakahlia	IBD	4.6**	4.8**	8.0
21	IBD	SD	Dakahlia	IBD	4.6**	4.8**	18.0
22	ND HitchnerB1	SD	Dakahlia	NDV	6.9	≥ 7.5	18.0
23	ND LaSota	SD	Dakahlia	NDV	≥ 7.5	≥ 7.5	7.6
24	ND LaSota	SD	Dakahlia	NDV	7.1	≥ 7.5	8.5
25	IB + ND Clone	SD	Fayoum	NDV	7.1	7.1	16.0
		SD	Fayoum	IBV	≥ 5.5	≥ 5.5	16.0
26	Pox + AE	PD	Dakahlia	POX	4	4.2	17.0
		PD	Dakahlia	AEV	3.7	3.7	17.0
27	IB + HitchnerB1	PD	Dakahlia	NDV	≥ 7.5	≥ 7.5	15.0
		PD	Dakahlia	IBV	≥ 5.5	≥ 5.5	15.0

ND = Newcastle disease; IBD = Infectious bursal disease; IB = Infectious bronchitis; AE = Avian encephalomyelitis; PD = Primary Distributor; SD = Secondary Distributor; AE = Avian encephalomyelitis virus; NDV = Newcastle disease virus; IBV = Infectious bronchitis virus; POX = Pox virus. *Egg infectious dose (EID) or tissue culture infectious dose (TCID) was used for evaluation; **TCID₅₀/dose.

Note: CLEBV samples are paired vaccines from the same batch as those collected from the field. They were previously stored as back-up following the evaluation of the samples to be released for farmer use. Storage temperature was taken at the same time the questionnaire was administered and does not reflect all the temperature ranges the vaccine have been subjected to previously.

Table III. Comparison of hemagglutination-inhibition arithmetic mean titers of field samples and retained stock samples.

S/no.	Vaccine	Source	Governorate	Virus	Log titer of the tested field sample (HI*)	Log titer of CLEVB sample (HI*)	Storage temperature of field samples (°C)
28	Locally produced iH5N1	PD	Dakahlia	AI_H5	5	8	17.0
29	Imported H5N1	PD	Sharqia	AI-H5	7	8.3	6.5
30	Imported H5N1	SD	Sharqia	AI-H5	8.25	8.5	5.3
31	Imported H5N1	SD	Sharqia	AI-H5	10	11	4.5
32	Imported ND	SD	Sharqia	NDV	6.5	6.5	15.0
33	Imported ND broiler	SD	Fayoum	NDV	7	7.5	8.9
34	Imported NDV	PD	Dakahlia	NDV	6.4	6.5	15.0
35	Imported ND	SD	Fayoum	NDV	6.25	6.75	7.0
36	Imported ND	SD	Fayoum	NDV	7.1	7.1	16.0
37	Locally produced H9+ND	PD	Sharqia	AI-H9	8.75	9.25	7.5
		PD	Sharqia	NDV	8.5	8.5	7.5

*HI = Arithmetic mean hemagglutination inhibition titerlog.; PD=Primary distributor; SD= Secondary distributor.

Table IV. Odds ratio of effective titres of the field vaccines against some variables using binary logistic regression.

Variable	Odds ratio	Standard error	P-value	95% Confidence interval
Optimum storage temperature	0.49	0.32	0.28	0.13; 1.78
Primary distributors	0.75	0.49	0.66	0.21; 2.68
Secondary distributors	1.33	0.87	0.66	0.37; 4.77

Pairwise correlation coefficients between effective titres of the field vaccines and optimum storage temperature, primary distributors and secondary distributors were -0.1705, -0.0692 and 0.0692, respectively.

effectiveness routinely, there was a likely failure due to intermittent break in cold chain as experienced during electricity interruptions and during vaccine evacuations to clean out the fridge.

Between 2006 and 2014, a total of 832 billion doses of avian influenza vaccine were released and used to control avian influenza outbreaks, yet the virus continues to circulate in the Egyptian poultry (CLEVB unpublished data). The failure to control outbreaks despite these efforts were associated, among others, with partial immunisation due to low quality vaccine, suboptimal dosages, improper vaccination, continuous shedding and silent transmission, possible mutation of the viruses, and field virus variants which escaped vaccine-induced immunity (Lee *et al.* 2004, Smith *et al.* 2006, Taha *et al.* 2007, Escoria *et al.* 2008, Domenech *et al.* 2009, Rudolf *et al.* 2010, Kilany *et al.* 2015). We have provided more revelations on the role of distribution chain.

While our 'on-the-study' survey of refrigerators' temperatures indicated that 61% of them failed to

keep optimum range, negative correlation existed with the measured titres. We concluded that a one-time evaluation of vaccine storage refrigerators may not be a good indicator to determine continuous maintenance of cold chain and assess the effectiveness of stored vaccines. It is highly likely that the stored vaccines were subjected to freeze-thawing during the process of cleaning and defrosting, and during electricity interruptions, and the study participants confirmed this hypothesis. Previous reports from Egypt have confirmed that vaccine failed to protect poultry effectively against challenge pathogens in the field (Kim *et al.* 2010, Arafa *et al.* 2012, Kilany *et al.* 2015).

Furthermore, because domestic refrigerators were designed and built for food and drink storage, they may have not met the special temperature requirements for vaccines. Purpose built vaccine refrigerators come with temperature regulation mechanisms that ensure narrow variations in internal temperatures, with an on-going air circulation system that ensures even distribution of temperature which should prevent vaccine from freezing². In the event of multiple opening and closing of refrigerators for purposes of sale, taking out of food and water, and during the process of cleaning and defrosting, there may be a shift in optimum temperature with implications for stored vaccines. In addition, because no defined procedure or record was established for cleaning and defrosting, and no record of where vaccines

² Ontario Ministry of Health and Long Term Care. Vaccine Storage and Handling Guidelines. GSIN: 7540-19600E May 2013. Queen's Printer for Ontario, 2013. http://www.health.gov.on.ca/en/pro/programs/publichealth/oph_standards/docs/reference/vaccine%20_storage_handling_guidelines_en.pdf.

were kept while these processes were on-going existed, vaccine immunogenicity, shelf life, potency and titres may have been affected.

We have also provided evidence of contaminant on the vial of vaccine sold in Egypt. Specifically, a lentogenic strain of ND virus was detected. Whether this is a vaccine virus (LaSota virus, MDT = 103 hours) or field virus was not established in this study, however, this contamination posed potential risk to vaccinated poultry. Because most of live poultry vaccines are used via drinking water, and the vials are immersed in the tanks before opening, it may become necessary to decontaminate the exterior of vaccine vials before use. Such decontamination must be followed by adequate rinsing to eliminate potential residual effect of decontaminants on vaccines.

Distributors, particularly the secondary ones, rarely give attention to personal hygiene and often place extraneous materials in the vaccine fridges. These activities increase the burden of contamination of vaccine vials. Approximately 74% of poultry production in Egypt remains with the small-scale producers who rely heavily on these small-scale secondary distributors for medicine and poultry vaccines (Ali *et al.* 2013). Because many of the poultry producers operate with low to moderate biosecurity in farms and most of their animal products end up in the live bird markets, we advocate for pre-slaughter screening and inspection for zoonotic viruses in poultry. The contribution of low quality vaccines, poor biosecurity measures and the consequent protection failure, disease outbreaks, economic losses, and potential silent spreading of pathogens continue to pose threat to human and animal health. Small-scale distributors must be trained to know their potential roles in poultry diseases transmission and how to conduct responsible storage and vaccine distribution chain. Such education may be facilitated by vaccine companies and commercial poultry associations.

This study was subjected to certain limitations: we assessed vaccinal and HI titres as a measure of vaccine efficacy and conducted live bird experimental vaccination where necessary but did not follow up with complete challenge studies. In addition, we established the loss in vaccinal titres and associated it with distribution chain but couldn't draw conclusion on the role of cold-chain because no correlations existed between the two. However, evidence from the distributors confirmed

repeated break in cold chain and previous workers demonstrated this association (Thakker and Woods 1992). Finally, we did not answer whether exposure to adverse high temperature or freezing, and the length of time of exposure actually damaged vaccine and affected its potency in Egypt. We advocate for a broader study to assess these limitations and possibly conduct experimental vaccination and challenge in a controlled environment.

Conclusions

While we acknowledged that vaccines were affected by a variety of factors, in Egypt, it would appear that viral vaccines of poultry were less effective and efficacious due to poor handling, break in cold chain and poor distribution networks. An overhaul of the vaccine value chain is necessary to improve poultry vaccine efficacy in Egypt.

Statement of animal rights

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