Comparison of the efficacy of local and imported inactivated combined H9-ND virus vaccines in protection of broiler flocks against H9N2 infection in Egypt

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ABSTRACT

This study was conducted to compare the efficacy of two combined H9-ND virus vaccines, one imported and the other local, through two experiments. The first experiment was conducted in the laboratory using 150 one day old chicks divided into 3 groups, the first two groups were vaccinated by the imported and local vaccines, respectively at one day old, the third group was kept as negative control. The antibodies against H9N2 virus were detected and measured by HI test at 14-, 21- and 28-days-post-vaccination. At 21 days, each vaccinated group was divided into two sub-groups; the 1ˢᵗ was kept unchallenged, and the 2ⁿᵈ was challenged with the circulating local H9N2 virus strain. In the chicken isolators, cloacal and tracheal swaps were taken at 2-, 4- and 6-days post-challenge to detect the shedding virus using real-time RT-PCR. The second experiment was in a commercial broiler flock contain 432,000 chickens placed in 16 pens. The broiler chickens were divided into 2 groups; each group received a different commercial H9-ND vaccine at the 1ˢᵗ day and the humoral immunity was measured at 14-, 21- and 28-days by HI test. The results showed that the locally produced vaccine provided a significant higher immune response in both lab. and field experiments and the virus shedding was stopped early (at 4th day post-challenge in cloacal swap) in the group vaccinated by local vaccine. In conclusion, the vaccine prepared from local isolate in Egypt is preferred than imported vaccine in the control of the Avian influenza

1. INTRODUCTION

Avian influenza (AIV) is a contagious viral disease, classified as a member of Orthomyxoviridae family where its genome is segmented, single strand negative sense RNA (Jordan et al., 2018). Avian influenza viruses is divided into three distinct types; A, B, and C based on serologic reactions to the internal proteins, principally NP and M1 proteins (Calnek, 1997). Avian influenza viruses are divided into subtypes based on the antigenic relationship in the surface glycoprotein into 18 hemagglutinin (HA) and 11 neuraminidase (NA) with variable combinations. AIV are further classified into two pathotypes known as a highly pathogenic avian influenza virus (HPAIV) and a low pathogenic avian influenza virus (LPAIV) (Capua and Alexander, 2004; Klenk et al., 2008; Saif, 2008).

HPAIV causes fatal systemic infection inducing high mortality up to 100% in all sectors of domestic bird’s broilers, layers and breeders while LPAIV produce asymptomatic infection (Capua and Alexander, 2004). Previous experimental study demonstrated that the inactivated vaccines are capable of inducing antibody response, which help in the protection of the infected birds from the clinical signs and mortalities (Capua and Alexander, 2008). H9N2 avian influenza virus have caused several outbreaks in poultry since 1990, resulting in high economic losses in Asia and Middle East (Das and Suares, 2007). In Egypt the virus is considered one of the major viral problem affecting the poultry industry since its first official reporting on 2010 till now (El-Zoghby et al., 2012). However, the experimental infection of non-specific-pathogen-free chickens with H9N2 virus did not show any clinical symptoms (Mo et al., 1998). Co-infection of H9N2 viruses with either bacteria such as Staphylococcus aureus and Haemophilus paragallinarum or with attenuated coronavirus causing serious disease (Haghhighat-Jahromi et al., 2008). Avian influenza virus infection causes high economic losses in both layers and breeder due to drop in egg production also, sever losses in broilers was reported specially in case of co-infection of other viral or bacterial diseases (Monne et al., 2013). Moreover, it was reported that H9N2 virus causes immunosuppression and alteration to the blood biochemical and hematological parameters in the poultry farms in Egypt (Sultan et al., 2015). The continuous genetic changes, either in the envelope or in the internal genes of the isolates, may result probably from the pressure of vaccination, or other unknown reasons (Banet-Noach et al., 2007; Golender et al., 2008; Perk et al., 2009).

The chicken immune system plays a critical role in broiler chicken performance as it reflects the health status of the broiler flocks. The different pathogens get recognized by special receptors in the vertebrate’s cells belong to the pathogen recognition receptors (Elfeil et al., 2012;
Abouelmaatti et al., 2013; Elfeil et al., 2016). The detection of H9N2 virus shed from the infected bird is an important tool for evaluation of the vaccine effectiveness, as the effective vaccine not only prevents clinical disease, but also reduces the viral shedding, to another susceptible birds (Subtai et al., 2011). The vaccine adjuvant plays a role in stimulation of the cellular immunity in case of inactivated vaccines. The aim of this study was to compare the effectiveness of two commercial H9-ND vaccines one imported vaccine (imported-1) and the other was produced locally from local circulating strain in Egypt.

2. MATERIAL AND METHODS

2.1. Birds of the lab. experiment:
A total of 150 one-day old divided into three groups. The 1st group (G1) took the locally prepared H9-ND vaccine. The 2nd group (G2) took the imported H9-ND at one day old by s.c injection according to the manufacture recommended dose (0.3 ml/bird for local vaccine and 0.2 ml/bird for imported vaccine). The 3rd group (G3) was kept as negative control.

The chicken were kept for 30 days with daily observation and about 10 blood samples from each group were collected at 14, 21 and 28 days. At 21 day old 25 chicken from each group were taken to the isolators for challenge with H9N2 virus by dose 0.1 ml per bird of 10^6 EID50 (Sultan et al., 2015). oropharyngeal and cloacal swaps were taken separately from each group at 2, 4 and 6 days post-challenge for detection of virus shedding using real time RT-PCR.

2.2. The challenge virus
The challenge virus is H9N2 virus [A/CK/Eg/114940v/NLQP/2011(H9N2)] (GenBank Q440373). The virus titer:10^6EID50. Dose: 0.1 ml per bird.

2.3. The primer of real time RT/PCR test:

Table1 The Experimental design
| Group No. | No. of Birds | Vaccination regime | Age/days | Dose/bird  | Challenge at 21 days of age | Assessment of protection |
|-----------|-------------|---------------------|----------|------------|-----------------------------|--------------------------|
| 1         | 25          | local               | One      | 0.3/bird   | ++                          | Shedding at 2,-4, and 6 days |
| 2         | 25          | imported            | One      | 0.2/bird   | ++                          | Shedding at 2,-4, and 6 days |
| 3         | 25          | Non vaccinated      | ---      | ---        | --                          | HI test                  |

**++**: challenged at 21 day old

2.7. Hemagglutination inhibition (HI) test:
The HI test used to monitor the post vaccination humoral immune response for each vaccine; using an avian influenza H9N2 antigen prepared Me-Vac Company (Salihya, Egypt) from an RLQP isolate (represented the circulating virus in Egypt). Chicken sera were examined for HA-specific antibodies against H9N2 virus by HI test according to OIE manual (OIE, 2005).

2.8. Challenge experiment:
At 21 day old 25 birds from each group were challenged in Biosafety Level three chicken isolators, at CLEV.

The challenged birds received a 10 μldose at10^50% egg infective dose (EID50)/10 μl via the intranasal route. At 2-4 and 6 days post-vaccination (DPC), cloacal and oropharyngeal swabs were collected from each challenged group separately for virus detection and titration by real time RT-PCR.

2.9. RNA extraction and real time RT-PCR:
Total RNA from cloacal and tracheal swabs collected from vaccinated challenged chickens from groups A and B, and from non-vaccinated challenged group using RNA
3. RESULTS

The HI test results of the lab. experiment

The geometrical mean of HI titers log2 in chicken measured at 14, 21, and 28 day of life. The antibodies mean titer at 14th day were (3.5 ± 0.31), (3.2 ± 0.31) and (1.0 ± 0.31) for G1, G2 and G3 respectively. At 21th day of life, the antibodies were (4.4 ± 0.21), (4.0 ± 0.21) and (0.8 ± 0.21) for G1, G2 and G3 respectively. At 28th day of life, the antibodies were (5.0 ± 0.41), (4.4 ± 0.41) and (0.0 ± 0.41) for G1, G2 and G3 respectively (Table 2).

The HI test results of the field experiment

The geometrical mean of HI titers log2 in chicken measured at 14, 21, and 28 days of life. The antibodies mean titers at 14th day of life were in the local vaccine was (3.6 ± 0.431), while the imported-1 vaccine mean antibody titer was (3.4 ± 0.431). The antibodies mean titers at 21th day of life were in the local vaccine was (4.3 ± 0.212), while the imported-1 vaccine mean antibody titer was (4.1 ± 0.212). At 28th day of life; the mean antibody titers in the locally produced vaccinated group was (5.2 ± 0.24); while the imported-1 vaccinated group was (4.4 ± 0.24)(Table 3).

Results of shedding

At 2nd day post challenge the virus was detected by high concentration in all groups, in both cloacal and tracheal swaps, at 4th day post-challenge the virus detected in both oropharyngeal and cloacal swaps of G2,G3 and only oropharyngeal swap of G1, at the 6th day post-challenge the virus detected only in non-vaccinated group, while both vaccinated groups were negative in cloacal and oropharyngeal swaps (Table 4).

4. DISCUSSION

AIV is one of the most important viral diseases in the industry of poultry and has a worldwide distribution (Stallknecht and Shane, 1988). A large numbers of outbreaks have occurred in Egypt since the first detection (El-Zoghby et al., 2012) these have caused severe health problems and large economic losses. In the present study we demonstrate that the inactivated vaccine either locally prepared or imported are capable of inducing antibody response which come in agreement with Bublot et al. (2007). Our results showed that the mean log titers at 14 and 21 days of life in the locally prepared H9N2 vaccinated group (prepared from circulating strain in Egypt) has a higher mean antibody titer than the imported-1 vaccine; but the difference were non-significant at this stage of life which logic at this early stage of life (Choi et al. 2008; Lee et al. 2011; Khalil et al. 2015).

Table 3 The antibodies mean titer of Imported-1 and locally produced H9N2 vaccine at 14, 21 and 28 day of life

| Group No. | Type of vaccine | G.M of HI titer log 2 at days post vaccination |
|-----------|----------------|---------------------------------------------|
| 1         | Local          | 3.6 ± 0.43                                 |
| 2         | Imported       | 3.4 ± 0.43                                 |
| 3         | Non vaccinated | 1.0 ± 0.31                                 |

*: day of life

Table 2 The antibodies mean titers of Imported-1 and locally produced H9N2 vaccine at 14, 21 and 28 day of life

| Group No. | Type of vaccine | G.M of HI titer log 2 at days post vaccination |
|-----------|----------------|---------------------------------------------|
| 1         | Local          | 3.5 ± 0.31                                 |
| 2         | Imported       | 3.2 ± 0.31                                 |
| 3         | Non vaccinated | 1.0 ± 0.31                                 |

*: day of life

Table 4 The virus shedding in oropharyngeal and cloacal swaps at 2-4and 6 days post challenge with H9N2 virus using real time RT-PCR

| Days post challenge | Group No. | G1 | G2 | G3 |
|---------------------|-----------|----|----|----|
|                     | Oropharyngeal | Cloacal | Oropharyngeal | Cloacal | Oropharyngeal | Cloacal |
| 2nd                 | CT > 1.9    | CT > 10   | CT > 1.9    | CT > 10   | CT > 1.9    | CT > 10   |
|                     | CT > 10    | CT > 10   | CT > 1.9    | CT > 10   | CT > 1.9    | CT > 10   |
| 4th                 | CT > 10    | CT > 10   | CT > 1.9    | CT > 10   | CT > 1.9    | CT > 10   |
|                     | CT > 10    | CT > 10   | CT > 1.9    | CT > 10   | CT > 1.9    | CT > 10   |
| 6th                 | CT > 10    | CT > 10   | CT > 1.9    | CT > 10   | CT > 1.9    | CT > 10   |

*: group
geneic and antigenic similarity to the circulating field strain which is coordinated with Wood et al. (1985). In addition, our results agreed with Kilany et al. (2016), who found that the titer of the virus shedding via oropharyngeal and cloacal swabs was lower in the vaccinated groups than the unvaccinated challenge one.

5. CONCLUSION
The vaccines prepared from local circulating virus in an area in Egypt provide better humeral immune response and low virus shedding which turn higher protection level against the suspected infection in the same area.

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