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Original Article

Immunogenicity and safety levels of pandemic influenza H7N9 vaccines in healthy individuals via meta-analysis

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Abstract: Objective: The aim of the current study was to evaluate immunogenicity and safety levels of human H7N9 influenza vaccines via meta-analysis. Methods: Searches were conducted in PubMed, Cochrane Library, ClinicalTrials.gov, and EMBASE databases. The purpose was to collect and perform meta-analysis of related randomized clinical trial (RCT) data concerning safety and immunogenicity levels of human H7N9 vaccines, published from the establishment of the database to September 2019, according to inclusion and exclusion criteria. Results: For non-adjuvanted human H7N9 vaccines, high doses were shown to induce limited immunogenicity. For adjuvanted human H7N9 vaccines, AS03, MF59, ISCOMATRIX, and Al $(OH)_3$ adjuvants, used in human H7N9 vaccines, can effectively increase immune response. Moreover, non-aluminum adjuvants performed better when using the same vaccine dosage. However, aluminum adjuvants demonstrated safety advantages in H7N9 adjuvants. Conclusion: Two doses of H7N9 vaccines, combined with an adjuvant, can achieve better immune effects. Adjuvanted H7N9 vaccines may ultimately be the best choice for pandemic H7N9 influenza.

Keywords: H7N9, vaccine, immunogenicity, safety, meta-analysis

Introduction

On March 31, 2013, the Health Department of China notified the World Health Organization (WHO) of 3 confirmed human cases of influenza A (H7N9) in Shanghai and Anhui. Afterward, there were five outbreaks of H7N9 bird flu [1], with an overall mortality rate of nearly 40% [2]. It has been found that, in general, human beings lack basic immunity to H7N9 avian influenza virus. There are no specific antibodies against H7N9 virus in the human body. Thus, vaccination is an important means of preventing H7N9 influenza viruses [3, 4]. At present, studies concerning safety and immune efficacy levels of human H7N9 vaccines are in the clinical trial stage. There are many sorts of vaccines, different doses, and different kinds of adjuvants [4]. It is difficult to accurately judge immune efficacy, optimal dosage, best vaccine form, and effects of the adjuvant on antibody titer of H7N9 vaccines using a single study. The current study performed a meta-analysis of published studies concerning safety and immunogenicity levels of human H7N9 vaccines and several adjuvants, providing an evidence-based conclusion for the use of H7N9 vaccines.

Materials and methods

Search resources and strategies

Databases, including Cochrane Library, PubMed, ClinicalTrials.gov, and EMBASE, were searched using the keywords influenza, H7N9, and vaccine. The retrieval time was from the establishment of the database to December 2019. Language was limited to English.

Inclusion and exclusion criteria

Inclusion criteria: (1) Randomized controlled trials, cohort studies; (2) Subjects that were normal people (age and ethnicity were not limited); (3) Interventions were vaccinated with H7N9 avian influenza vaccine (no restriction on vaccine types); (4) Control measures were blank control, self before-and-after control, or intergroup control; and (5) Outcome indicators were

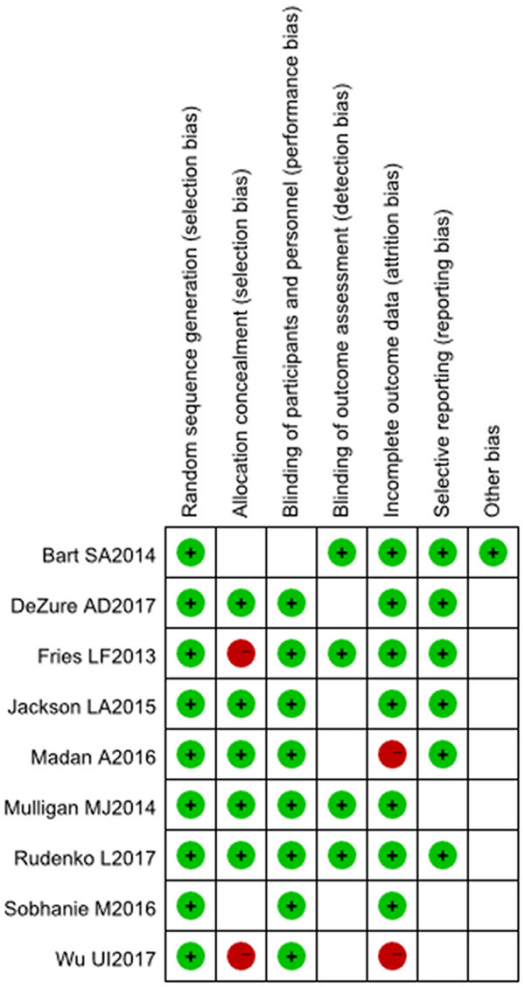

Figure 1. Diagram of risk of bias summary for the 9 eligible studies.

H7N9 virus infection rates, incidence of influenza-like symptoms, seroconversion rates (SCR), and so forth. Exclusion criteria: (1) Animals, pregnant and lying-in women, breast-feeding women, patients suffering from diseases affecting immune function or uncontrollable chronic diseases; (2) Subjects that had been vaccinated with H7N9 or H7 influenza vaccine before. This may have an impact on the immune effect; (3) Republished studies, irrelevant studies, conference abstracts, reviews, mechanism studies, and research studies irrelevant to the immune effects of H7N9 avian influenza vaccines.

Literature screening and quality evaluation

Two researchers, independently, read the abstracts and titles for screening. They then selectively read the full texts, according to inclusion and exclusion criteria. Cochrane's Randomized Controlled Trials Bias Assessment Tool was used to evaluate study quality [5]. Risk of bias assessment contained 7 items, including the method of generating random sequence, process of allocation concealment, incomplete outcome data, blinding of outcome assessment, blinding of participants and personnel, selective reporting, and other bias. The two researchers discussed divergences in literature inclusion and quality evaluation, consulting a third person if necessary.

Data extraction

The two researchers extracted all relevant data, independently entering the information into a specially designed data extraction table. Data extraction included the first author, publication time, sample size, age of subjects, type of vaccines (dosage, dose and adjuvant type), outcome indicators, and study design.

Statistical analysis

The database was collected using Microsoft Excel. RevMan 5.3 software was used for meta-analysis. Evaluating immunogenicity and safety levels of H7N9 vaccines, the current study used the Mantel-Haenszel (M-H) method (random effects model), calculating the 95% confidence interval (95% CI) and risk ratio (RR) of the data. I^2 statistics was applied to assess statistical heterogeneity. Differences between adjuvant and non-adjuvant vaccines were compared. Adjacent vaccine dose groups with or without adjuvants were also compared. Publication bias was evaluated using RevMan 5.3. Results are shown in the risk of bias summary diagram (**Figure 1**).

Results

Basic features and quality evaluation of included studies

A total of 534 studies were retrieved. Six studies [6-11] were finally included, all written in English. The process of literature selection is shown in **Figure 2**. Each of the six studies concerned human inactivated H7N9 avian influenza vaccines. Serological indicators were used to assess immune effects. Outcome indicators were SCR measured by HI before and after vaccination (i.e. the percent of the population serum titer before vaccination ≤ 1:10 and se-

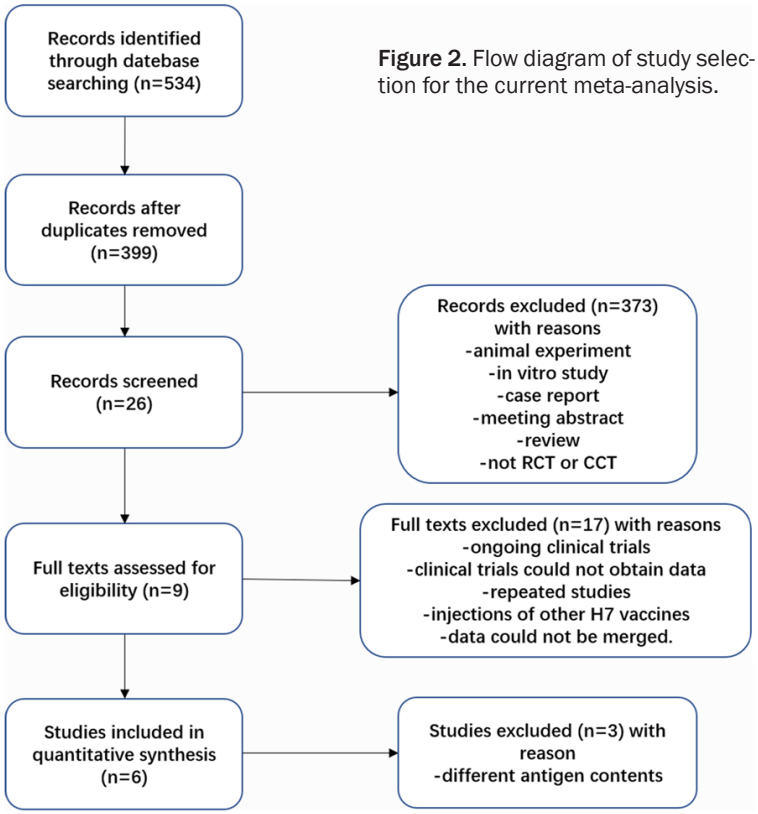

Figure 2. Flow diagram of study selection for the current meta-analysis.

rum antibody titer ≥ 1:40, 3-4 weeks after vaccination). Basic information of included studies is shown in **Table 1**.

Meta-analysis of immunogenicity in different doses of H7N9 vaccines

According to intervention measures included in the current study, there were a total of 7 groups. These included the 3.75 μg+ adjuvant group, 5 μg+ adjuvant group, 7.5 μg+ adjuvant group, 15 μg+ adjuvant group, ≥ 30 μg+ adjuvant group, 15 μg without adjuvant group, and ≥ 30 μg without adjuvant group. RD values of SCR, before and after inoculation, were calculated using a random effects model. Results showed that, after 1 dose of the inoculation, RD values of SCR in each group were 1%-10%. Immune effects were poor. After 2 doses of inoculation, RD values of the SCR without adjuvant group were 11%-18%, still not playing a protective role. RD values of the SCR with adjuvant group were 52%-73%. These results met the approved standard of influenza vaccines by American Biological Products Evaluation and Research Center. The licensing criteria (SCR ≥ 40% for people aged 18~65 years old and SCR ≥ 30% for people aged ≥ 65 years old) was met [13] and all had good immunogenicity in the whole dosage range, as shown in **Table 2**. In the adjuvant vaccine groups, there were no significant differences in RR values of SCR between adjacent dose groups (3.75 μg vs. 7.5 μg; 7.5 μg vs. 15 μg) (P>0.05). However, adjacent dose groups (5 μg vs. 15 μg; 15 μg vs. 30 μg) showed significant differences (P ≤ 0.01), as shown in **Figure 3**. In general, for human H7N9 vaccines, high doses can induce limited immunogenicity.

Meta-analysis of immunogenicity of H7N9 vaccines with or without adjuvants

The adjuvant could be a significant factor in the use of vaccines. Using adjuvants in vaccines can increase the immune response, inducing effective antibodies and minimizing vaccine doses.

Comparison results of the immunogenicity between adjuvanted and non-adjuvanted H7N9 vaccines through the present meta-analysis are shown in **Figure 4**. Compared with 15 μg HA without aluminum adjuvant, the group of 15 μg HA+Al $(OH)_3$ adjuvant did not increase immunogenicity significantly (95% CI (0.56, 1.56), RR=0.94 determined by HI method). In contrast, other adjuvanted vaccines, such as ISCOMATRIX, MF59, and AS03, showed significantly higher immunogenicity levels than those of 15 μg HA without adjuvants (AS03 adjuvant: 95% CI (10.04, 156.65), RR=39.66; MF59 adjuvant: 95% CI (6.30, 28.26), RR=13.34; ISCOMATRIX adjuvant: 95% CI (3.23, 22.99), RR=8.61, measured by HI assay). It should be noted that immunogenicity levels of the 30 μg HA+Al $(OH)_3$ adjuvant vaccine were better than those of 30 μg HA without aluminum adjuvant (95% CI (0.96, 1.98), RR=1.38 by HI assay). Present results suggest that application of these 3 adjuvants (ISCOMATRIX, MF59, and AS03) in human H7N9 vaccines could increase overall immune response significantly. In sum-

Table 1. Characteristics of eligible trials

First Author	Published Year	Vaccine Type	Adjuvant Type	Design	HA Antigen Dosage	Main Outcome
Bart SA [6]	2014	inactivated subunit	MF59	RCT	3.75 μg, 7.5 μg, 15 μg	HI, MN, AE
DeZure AD [20]	2017	DNA or inactivated subunit (prime)		RCT	4 mg or with 45 μg (prime)	HI, MN, AE
		inactivated subunit (boost)			45 μg (boost)	
Fries LF [7]	2013	VLP	ISCOMATRIX	RCT	placebo, 5 μg, 15 μg, 45 μg	HI, AE
Jackson LA [8]	2015	inactivated split	MF59, AS03	RCT	3.75 μg, 7.5 μg, 15 μg, 45 μg	HI, MN, AE
Madan A [9]	2016	inactivated split	AS03	RCT	placebo, 3.75 μg, 7.5 μg, 15 μg	HI, AE
Mulligan MJ [10]	2014	inactivated split	MF59	RCT	3.75 μg, 7.5 μg, 15 μg, 45 μg	HI, MN, AE
Rudenko L [21]	2017	LAIV		RCT	placebo, 7.5 $log_{10}EID_{50}$	HI, MN, AE
Sobhanie M [22]	2016	LAIV (prime)		RCT	$10^{7.0}$ FFU (prime)	HI, MN, AE
		inactivated sub-virion (boost)			30 μg (boost)	
Wu UI [11]	2017	inactivated whole-virion	Al $(OH)_3$	RCT	15 μg, 30 μg	HI, MN, AE

Table 2. Comparison of SCR in different doses of H7N9 avian influenza vaccine groups

Groups	Number of studies	First dose				Second dose			
		Sample size	*P* value	RD (%)	95% CI (%)	Sample size	*P* value	RD (%)	95% CI (%)
3.75 μg+ adjuvant	4	403	0.16	6	[-2, 13]	400	<0.01	66	[36, 95]
5 μg+ adjuvant	1	73		0	[-0.03, 0.03]	73		73	[62, 83]
7.5 μg+ adjuvant	4	403	0.2	10	[-5, 25]	403	<0.01	69	[44, 94]
15 μg+ adjuvant	6	695	0.21	3	[-1, 6]	694	<0.01	52	[38, 66]
≥ 30 μg+ adjuvant	1	49		10	[1, 19]	48		65	[51, 78]
15 μg	6	433	0.06	1	[0, 3]	432	0.008	11	[3, 20]
≥ 30 μg	4	276	0.04	3	[0, 5]	275	0.02	18	[3, 33]

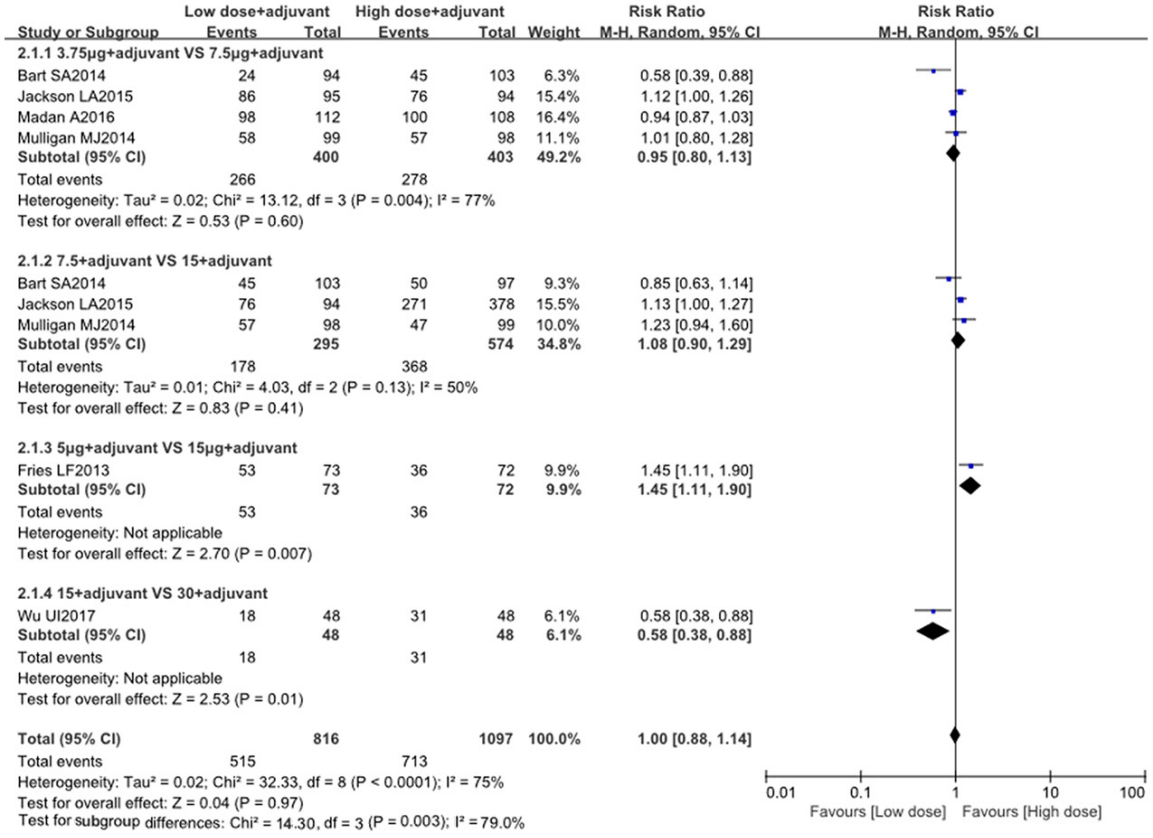

Figure 3. Comparison of SCR of antibodies against H7N9 in individuals between adjacent doses of adjuvanted inactivated H7N9 vaccines by HI assay after second vaccination.

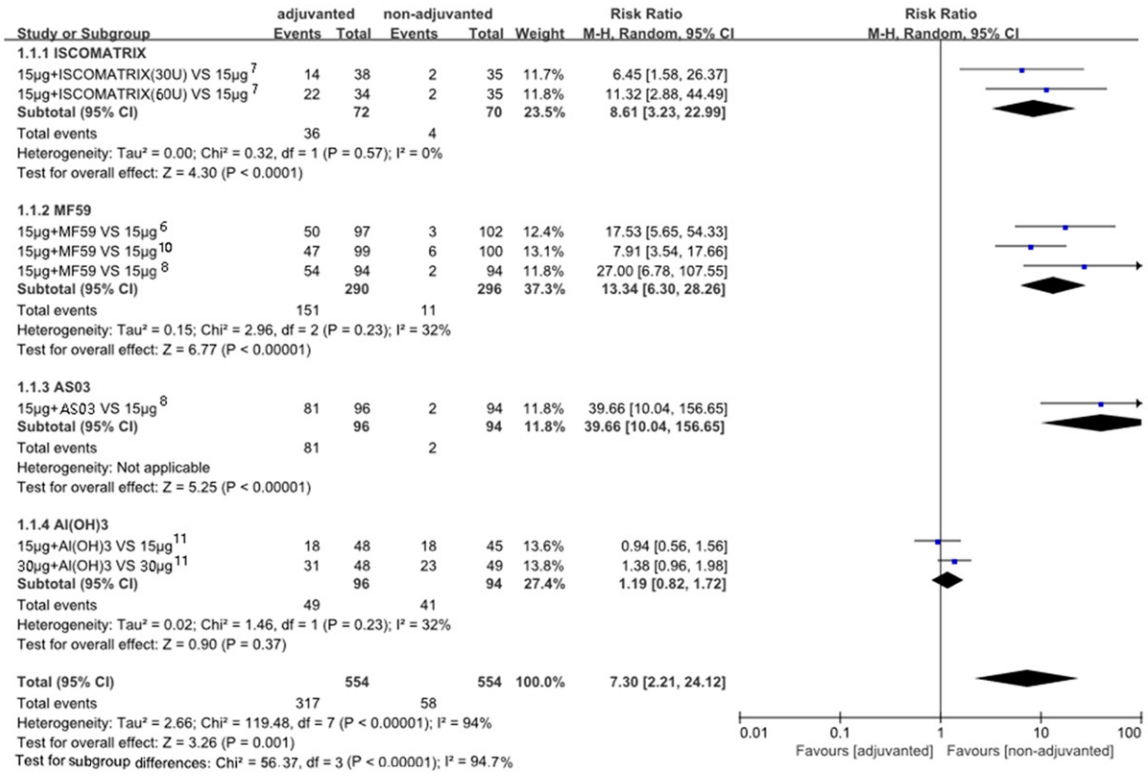

Figure 4. Comparison of immunogenicity between adjuvanted and non-adjuvanted human H7N9 influenza inactivated vaccines of different adjuvant types (ISCOMATRIX, MF59, AS03, and Al $(OH)_3$) detected by HI assay after 2nd vaccination.

mary, for adjuvanted human H7N9 vaccines, AS03, MF59, ISCOMATRIX, and Al $(OH)_3$ adjuvants used in human H7N9 vaccines can effectively increase overall immune response, compared with non-adjuvanted ones. Moreover, non-aluminum adjuvants performed better when using the same vaccine dosage.

Meta-analysis of the safety of H7N9 influenza vaccines with or without adjuvant

Meta-analysis results of systemic and local adverse events (AE), comparing human H7N9 vaccines with or without adjuvants, are shown in **Table 3**. Compared with vaccines without adjuvants, AS03-adjuvanted vaccines and ISCOMATRIX-adjuvanted vaccines increased the risk of systemic and local AE. Vaccines with MF59 adjuvant only significantly increased the risk of local AE. Unlike the above 3 adjuvants, vaccines with Al $(OH)_3$ adjuvant only slightly increased the risk of systemic and local AE, compared with vaccines without an adjuvant. In summary, present results suggest that the overall safety of Al $(OH)_3$ adjuvant was better than the other 3 adjuvants (MF59, AS03, ISCOMATRIX) applied in H7N9 vaccines.

Discussion

In recent years, with the prevalence of avian influenza viruses, such as H7N9 and H5N1, related vaccines have entered clinical trials. Generally, serological conversion of H5N1 non-adjuvant or aluminum adjuvant vaccines can only be achieved after 2 doses of high-dose inoculation [12]. Low doses of oil-in-water adjuvant can obtain good immune effects [13]. Similar to H5N1 vaccines, studies have found that the hemagglutinin of H7 subtype influenza virus has poor immunogenicity and that the protective effects of the vaccine can only be achieved through enhanced immunization and combined use of adjuvants [14, 15].

Table 3. Comparison of systemic and local AEs between non-adjuvanted and adjuvanted human H7N9 vaccines

Outcome or Groups	Studies [n]	Statistical method	Effect size
Systemic AE			
ISCOMATRIX adjuvant			
15 μg+ISCOMATRIX (30 U) VS 15 μg	1 [7]	Risk Ratio (M-H, Random, 95% CI)	1.40 [0.95, 2.07]
15 μg+ISCOMATRIX (60 U) VS 15 μg	1 [7]	Risk Ratio (M-H, Random, 95% CI)	1.74 [1.22, 2.48]
Subtotal (I-squared=0%, P=0.43)			1.58 [1.21, 2.05]
MF59			
15 μg+MF59 VS 15 μg	1 [6]	Risk Ratio (M-H, Random, 95% CI)	1.45 [1.07, 1.96]
15 μg+MF59 VS 15 μg	1 [8]	Risk Ratio (M-H, Random, 95% CI)	0.81 [0.62, 1.06]
15 μg+MF59 VS 15 μg	1 [10]	Risk Ratio (M-H, Random, 95% CI)	1.53 [0.99, 2.38]
Subtotal (I-squared=81%, P=0.006)			1.19 [0.78, 1.84]
AS03 adjuvant			
15 μg+AS03 VS 15 μg	1 [8]	Risk Ratio (M-H, Random, 95% CI)	1.67 [1.34, 2.08]
Subtotal (I-squared=%, P=.)			1.67 [1.34, 2.08]
Al $(OH)_3$ adjuvant			
30 μg+Al $(OH)_3$ VS 30 μg	1 [11]	Risk Ratio (M-H, Random, 95% CI)	1.38 [0.90, 2.10]
15 μg+Al $(OH)_3$ VS 15 μg	1 [11]	Risk Ratio (M-H, Random, 95% CI)	1.16 [0.77, 1.76]
Subtotal (I-squared=0%, P=0.58)			1.26 [0.94, 1.70]
Local AE			
ISCOMATRIX adjuvant			
15 μg+ISCOMATRIX (30 U) VS 15 μg	1 [7]	Risk Ratio (M-H, Random, 95% CI)	1.67 [1.08, 2.58]
15 μg+ISCOMATRIX (60 U) VS 15 μg	1 [7]	Risk Ratio (M-H, Random, 95% CI)	2.06 [1.37, 3.09]
Subtotal (I-squared=0%, P=0.57)			1.87 [1.39, 2.51]
MF59			
15 μg+MF59 VS 15 μg	1 [6]	Risk Ratio (M-H, Random, 95% CI)	1.43 [0.97, 2.09]
15 μg+MF59 VS 15 μg	1 [8]	Risk Ratio (M-H, Random, 95% CI)	1.49 [1.21, 1.84]
15 μg+MF59 VS 15 μg	1 [10]	Risk Ratio (M-H, Random, 95% CI)	1.41 [1.10, 1.83]
Subtotal (I-squared=0%, P=0.95)			1.45 [1.25, 1.69]
AS03 adjuvant			
15 μg+AS03 VS 15 μg	1 [8]	Risk Ratio (M-H, Random, 95% CI)	1.99 [1.64, 2.43]
Subtotal (I-squared=%, P=.)			1.99 [1.64, 2.43]
Al $(OH)_3$ adjuvant			
30 μg+Al $(OH)_3$ VS 30 μg	1 [11]	Risk Ratio (M-H, Random, 95% CI)	1.08 [0.77, 1.50]
15 μg+Al $(OH)_3$ VS 15 μg	1 [11]	Risk Ratio (M-H, Random, 95% CI)	1.12 [0.83, 1.52]
Subtotal (I-squared=0%, P=0.85)			1.10 [0.88, 1.38]

Inactivated vaccines are the most widely used type of influenza vaccines [16, 17]. Through a meta-analysis of 6 inactivated H7N9 avian influenza vaccines, it was found that the immune effects of each dose group after the 1st dose were poor. RD values of SCR were 1%-10%. After inoculation with the second dose, RD values of SCR without an adjuvant were 11%-18%, not achieving effective serological protection. In contrast, adjuvanted vaccine groups were able to achieve good results in the whole dose range. RD values of SCR were 52%-73%, meeting vaccine licensing standards. Results suggest that adjuvants play an important role in enhancing vaccine immunogenicity. One study [11] used an aluminum adjuvant. They found that the immune effects increased with the increase of vaccine dosage, consistent with the conclusion of MANZOLI [18] concerning immune effect analysis of H5N1 aluminum adjuvant vaccine. Effects of oil-in-water adjuvant (MF59 or AS03) vaccines were opposite to those of aluminum adjuvant vaccines. Immune effects of the lowest dose of 3.75 μg were com-

parable to those of the high dose of 15 μg. There were no significant differences between high dose and low dose groups. Through comparative analysis, it was found that the immune effects of AS03-adjuvanted vaccines performed better than those of MF59-adjuvanted vaccines and ISCOMATRIX-adjuvanted vaccines, in accord with the meta-analysis conclusion of GUO [19] on H5N1 vaccines.

The present study also found that incidence of adverse reactions after vaccinations was low, while vaccine safety was relatively high. Thus, it could be used for vaccination of high-risk groups. Using adjuvants in H7N9 vaccines can enhance the immunogenicity by inducing effective antibodies. Additionally, adjuvants can minimize the use of vaccine doses. However, adjuvants were shown to increase the risk of AEs, as shown in **Table 3**. Of the 4 adjuvants (AS03, MF59, Al $(OH)_3$, and ISCOMATRIX), the aluminum adjuvant showed better safety levels than the others when applied in H7N9 vaccines.

The best indicators to evaluate the immune effects of vaccines are infection rates and vaccine protection rates after vaccination. The current study only indirectly reflects the immune effects of vaccines through serological indicators, producing limitations. In addition, the number of studies included and overall sample sizes were small. Thus, efficacy levels of the tests may be insufficient. More relevant studies should be accumulated, further evaluating safety and immunogenicity levels of the human H7N9 influenza vaccine.

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Disclosure of conflict of interest

None.

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