Title: Vaccine Efficacy Against Indonesian Highly Pathogenic Avian Influenza H5N1: Systematic Review and Meta-Analysis

Article Type: Review Article

Keywords: Vaccine; Vaccination; Efficacy; Avian influenza; HPAI

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Abstract: Indonesia has implemented multiple strategies to control Highly Pathogenic Avian Influenza H5N1 (HPAI/H5N1), including the licensure and use of multiple vaccine formulations. The continuous drift of Indonesian HPAI/H5N1 viruses and emergence of a new clade in 2012 that became dominant in 2016, demands the assessment of commercial vaccine formulations against Indonesian field viruses.
Seven databases were explored to identify relevant literature reporting the performance of commercial vaccines against Indonesian HPAI/H5N1 viruses. After methodological assessment, data were collated and analyzed to report immunogenicity and vaccine efficacy (VE) to prevent respiratory and cloacal viral shedding 2-day post challenge, and death at the end of the follow-up period. Meta-analyses were performed to assess VE consistency of alternative formulations and to explore potential sources of heterogeneity in VE.
In total, 65 studies and 46 vaccine formulations from 13 articles were grouped per OIE's VE protocols (group 1) and variations of it (groups 2,3,4). We found that antigenic closeness between vaccine-seed and challenge virus might be a better proxy of VE than current estimates based on vaccine-homologous HI antibody titers, particularly against current fourth order clade viruses (groups 1&2). Prime-boosting was efficacious across different chicken breeds (group 3), and early vaccination may increase the risk of death (group 4). One Indonesian vaccine was tested against the new dominant clade, conferring consistent protection in chickens but not in ducks. Meta-analyses revealed high inconsistency ($I2 \geq 75\%$) and inefficacy of LPAI formulations against current field viruses, while potential sources of inconsistent VE were formulation of seed-homologous vaccines and the species vaccinated.
We conclude that the VE of commercial vaccines in Indonesia changes as Indonesian HPAI/H5N1 evolve into new clades, which should warrant continuous matching between vaccine-seeds and emerging HPAI/H5N1. Furthermore, given the characteristics of the new Indonesian dominant HPAI/H5N1 clade, further studies to confirm VE across species are warranted.

Vaccine Efficacy Against Indonesian Highly Pathogenic Avian Influenza H5N1: Systematic Review and Meta-Analysis

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Conflicts of interest

None.

Funding

Juan P. Villanueva-Cabezas conducts his PhD with the support of CONICYT (Government of Chile) through the program 'Becas Chile' and the NHMRC Centre of Research Excellence in Policy Relevant Infectious Diseases Simulation and Mathematical Modelling – PRISM 2 (GNT1078068).

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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[1]INTRODUCTION

Highly Pathogenic Avian Influenza H5N1 (HPAI/H5N1) is a transmissible disease that causes substantial morbidity and mortality in chickens [1]. Although zoonotic spillover remains a rare event, the ongoing reporting of human cases [2] makes the virus of public health importance.

Initial efforts to control HPAI/H5N1 in Indonesia were based on *stamping out* [3]; but these were unsuccessful. In 2004, the Government of Indonesia adopted a vaccination program to control the spread of HPAI/H5N1 among production systems with limited biosecurity, including most free-range, backyard, and semi-commercial systems [4]. The high cost and logistic difficulties of vaccinating these poultry, led to a program reformulation in 2006-2007 shifting the emphasis to semi-commercial flocks, designated by FAO as sector 3, in areas of high infection risk [5, 6]. The Indonesian vaccine program was implemented by licensing multiple vaccine formulations produced overseas and by promoting the production of vaccines by local companies [6]. It has been suggested that unlicensed vaccine might have been used in Indonesia [6, 7] favoring the emergence of new antigenically distinct HPAI/H5N1 clades [7].

Accurate assessment of efficacy of commercial vaccines against Indonesian circulating strains is essential, because the antigenicity of virus lineages that become enzootic tend to drift away from the original virus [8]. Per the OIE's manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE's manual) [1], the hemagglutination inhibition test (HI) is the standard for

[1] Abbreviations: A/turkey/Wisconsin/1968: **Wis68**; A/turkey/England/N28/1973: **Eng73**; A/duck/Potsdam/1402/1986: **Potsdam86**; A/chicken/Mexico/232/1994: **Mex94**; A/chicken/Legok/2003:**Legok03**; A/chicken/West Java/Pwt-Wij/2006: **Pwt06**; A/chicken/Indonesia/7/2003: **Indo03**; A/chicken/West Java/Smi-Hamd/2006: **Smi-Hamd06**; A/chicken/West Java/Smi-Mae/2008: **Smi-Mae08**; A/chicken/Purwakarta-Cilingga/142/2010: **Cillingga10**; A/chicken/Papua/TA5/2006: **Papua06**; A/chicken/West Java/Smi-Pat/2006: **Smi-Pat06**; A/chicken/West Java-Subang/029/2007: **Subang07**; A/duck/Sukoharjo/Bbvw-1428-9/2012: **Suko12**. Haemaglutinin antigen expressed in a recombinant fowlpox virus (FPV)-vectored vaccine (Trovac ™ - AI H5, Merial): A/swan/Hungary/499/2006: **HVTvect06;** Haemaglutinin antigen expressed in a recombinant herpesvirus of turkeys (HVT)-vectored vaccine (Vectormune® HVT AI, Ceva-Biomune) A/turkey/Ireland/1378/1983: **FPvect83**; Reverse genetics-generated vaccines: A/goose/Guangdong/1/1996: **RG-Guang96**; A/duck/Vietnam/C57/2004: **RG-Viet04**; A/chicken/Legok/2003: **RG-Legok03**.

assessing immunogenicity and potential efficacy of vaccines against this agent. The OIE's manual suggests that vaccine-induced seroprotective levels equivalent to geometric mean titers (GMT) ≥ 32 might prevent mortality after viral infection, while antibody levels equivalent to GMT ≥ 128 might reduce viral replication and shedding [1]. It is relevant to note though, that vaccine immunogenicity is generally estimated using the vaccine homologous antigen [7], which may be antigenically distant to the circulating viral strain.

While HI seroprotective titer is our best proxy measure of likely vaccine efficacy (VE), bridging studies demonstrating the relationship between threshold titers and clinical outcomes of importance (reduced viral shedding and mortality) are fundamental. Such studies inform strategies to reduce the economic losses caused by HPAI/H5N1 but most importantly, to reduce the threat that this agent poses for both animal and public health. Thus, following the guidelines for reporting systematic reviews in veterinary medicine [9], the objective of this work is to answer the question: *'what is vaccine efficacy of commercial monovalent vaccine formulations to prevent viral shedding and mortality in healthy domestic poultry infected with Indonesian HPAI/H5N1?'*

METHODS

Search Strategy

Seven key databases for veterinary science, Medline (Web of Science), Medline(Ovidsp), CABI, BIOSIS, Web of Science (Core Collection), Scopus, and Embase were explored to identify relevant scientific literature published up to March, 17th, 2016. Search terms were included in search strings addressing population, disease, intervention, evidence of infection, and location (Table S1). Articles returned by each search string were combined to produce a list of publications for each database. Lists were imported to endnote [10] for consolidation, de-duplication, and storage.

Relevance Screening, Inclusion Criteria, and Quality Assessment

JVC and MC conducted independent unblinded screening of titles and abstracts. An article was deemed relevant if: 1) it was peer-reviewed; 2) it described a primary research study; 3) it described an intervention using a commercial vaccine; 4) vaccination was applied to healthy domestic poultry; 5) VE was evaluated against an Indonesian HPAI/H5N1 virus; 6) it included a control group, and 7) it reported an outcome that allowed estimation of VE as defined in the research question. When title and abstract were insufficient to judge relevance, articles were retained for full text assessment. Hand-search of citations in the reference list of relevant articles was performed to identify other relevant publications missed by the search strategy.

The inclusion criteria were developed *a priori*. Articles with English title and abstract but written in a different language were retained and the corresponding author consulted for an English version; if this was not available, the article was translated using Google Translate (https://translate.google.com.au/), and the help of a native speaker. To be included, articles had to report a randomized controlled trial, controlled trial, or challenge study as these study designs allow assessment of VE [11] and report outcomes including seroprotective levels after vaccination, viral shedding, or mortality after challenge with an Indonesian HPAI/H5N1. When articles reported more than one trial or challenge study, only those that met the inclusion criteria were included in the systematic review. A methodological quality assessment of articles was performed using the risk of bias tool (RoB) [12], following the approach recommended by Sargeant and O'Connor [11, 13].

Articles or studies reporting on experimental vaccines, multi-seed vaccine formulations, heterologous prime-boosting, vaccine effectiveness, or any other aspect of vaccines and

vaccination of poultry against Indonesian HPAI/H5N1, were excluded as these were beyond the scope of the defined research question.

Data extraction and Statistical Analysis

Relevant data (Table S2) were collated using Microsoft Excel® and were organized for comparison per challenge clade, vaccine seed, and poultry species. None of the authors listed in the articles were approached for further clarification of these data.

Seroprotective levels after vaccination were recorded as GMT. Raw viral shedding data were used to estimate crude risk ratios (RR) and 95% confidence interval (CI 95%) that estimate the relative risk of viral shedding two day-post challenge (dpc) of vaccinated versus control birds. Likewise, RR and CI 95% were estimated using raw data on mortality at 2dpc, and at the end of the follow-up period. When a publication reported a single vaccine formulation against the same challenge virus more than once, we followed the Cochrane collaboration's approach [14] adding the sample size and number of events in the vaccinated and control group, respectively, to then estimate RR and 95%CI as before. When no events were reported, a small continuity correction factor of 0.5 was added to allow calculation of RR [14, 15]. VE, defined as the relative risk reduction of viral shedding, or the relative risk reduction of mortality after viral challenge in vaccinated birds compared to those in the control group, was calculated as (1 - RR).

Meta-Analysis

We used meta-analysis to explore: a) the VE consistency of commercial vaccine formulations against Indonesian viral challenges; and b) the VE consistency of available commercial formulations across species. VE heterogeneity was further explored in subgroups [16] restricting the meta-analysis to explicitly alternative seed-homologous vaccine formulations within a publication (different name or ID), against a common challenge virus, tested in poultry with the same characteristics. Meta-analyses were performed using a fixed-effect model [17]; estimates of

heterogeneity were taken from a Mantel-Haenszel model as recommended for trials that report few or zero clinical events [14]. Consistency was measured using I^2 as this is less affected by power issues than heterogeneity tests [16], a fundamental feature given the small size of efficacy studies in poultry. Pooled RR, 95%CI, and VE were estimated using the *metan* routine [18] in Stata v.12 [19].

RESULTS

Scope of the study and characteristics of publications included

Figure 1 summarizes the flow of search, screening, and selection of articles. All publications included were challenge studies: seven to assess vaccine immunogenicity and efficacy [7, 20-25]; four to explore transmissibility of AI among vaccinated birds [26-29]; and two to assess a "Differentiate infected and vaccinated animals" (DIVA) strategy [30, 31]. Table 1 summarizes relevant features of these publications.

In total, 65 challenge studies (hereinafter 'studies') that involved 46 vaccine formulations were reported. Vaccination was generally administered as per Product Information; however, in four publications [25, 27, 28, 30] it is unclear if this was the case as a specific dose per inoculation was reported. Primary immunization, and prime-boosting of two and three doses were reported in ten, three, and one publication, respectively.

Vaccine immunogenicity, assessed using HI test against the vaccine-seed and/or the challenge virus specific antigen, was available in all but one publication [26]. Inoculation of challenge viruses occurred the same day the vaccine immunogenicity was measured; the challenge dose was 10^6 EID_{50} per bird except in three studies in [26] and the studies in [31] that inoculated 10^5 EID_{50}. Both doses were lethal for the control group at the end of follow-up. Viral shedding was determined through oral and/or cloacal swabbing followed by viral isolation, titration in eggs, or PCR, performed under standard methods described in each publication. All publications reported

oral and cloacal viral shedding 2dpc except [21, 22] that reported shedding 3dpc. Viral shedding data reported in [24] and [31] was not suitable for analysis in this review. Survivorship was assessed for 7, 10 or 14 dpc; five publications reported mortality 2dpc [7, 25, 26, 28, 30] and all, except [30], reported mortality at the end of the follow-up period. Further details are in supplement 2.

The methodological assessment focused solely on the systematic risk of bias that would affect our evaluation of VE (Table S3). None of the publications reported random sequence generation. In three publications [25, 29, 30] birds could have been identified before allocation. Because vaccines were commercial and vaccination and outcome assessment followed standard procedures, all publications had low risk of performance and detection bias. Incomplete reporting of mortality was detected in [30], while in [28] and [31] outcome data were aggregated. Other potential sources of bias were found in [20], where vaccine-seeds were not identified; in [24], where the number of chickens was not reported; and in [29] where the immune status of commercial chickens in the vaccine group was not reported. Overall, articles had low to moderate risk of bias.

VE based on outcome measures.

Studies with similar characteristics were grouped and vaccine formulations assigned a unique identifier (v.1, v.2, etc.) to facilitate comparison of performance within and across figures (Table 1). VE against respiratory viral shedding 2dpc and death at the end of the follow-up period for groups 1 and 2 are in Figures 2 and 3; Figures for groups 3 and 4 and full analysis of outcomes for all groups are in Figures S1 and S2, and Table S2 (supplementary material).

Group 1 (Figure 2) is characterized by studies that closely follow OIE's standards, i.e., performed primary vaccination of 3-weeks old SPF chickens, followed by viral challenge 3wpv,

and birds were followed-up 14dpc to assess survivorship. Two studies in which primary vaccination was administered at 4 (v.13) and 16 (v.33) weeks of age, with viral challenge 2wpv, were also included here. Low Pathogenic Avian Influenza (LPAI) formulations induced seroprotective GMT >128, while Indonesian and reversed-genetic formulations induced GMT >32. These seroprotective levels were associated with efficacy ≥ 67% to reduce the number of respiratory shedders 2dpc with Smi-Hamd06 (clade 2.1.1), Papua06 (clade 2.1.3.1), and Suko12 (clade 2.3.2.1). Four LPAI formulations did not protect from respiratory viral shedding 2dpc with Indo03 (clade2.1) (v13, 26) and Pwt06 (clade 2.1.3.2) (v.9, 19). When protection against death was evaluated, LPAI, Indonesian, and reversed-genetic formulations were efficacious 14dpc with SMI-Hamd06, Papua06, and Suko12; also, LPAI formulations were efficacious preventing death in birds challenged with Indo03. Eleven formulations were not efficacious against death: six LPAI (v. 9-11, 18, 19, 25) and five reversed-genetics formulations (v.34-38) did not protect birds challenged with Pwt06.

The studies in group 2 (Figure 3) diverged from OIE's protocol by being conducted in commercial layer chickens, in which primary vaccination was administered at 3 or 4-weeks of age, followed by viral challenge 3 or 4wpv, and birds followed-up 14dpc. Two studies in which 3-weeks old Mojosari ducks were vaccinated, with viral challenge 3wpv, were also included in this group (v.29, 30). Few vaccines reported vaccine homologous HI titers and all, except one, reported challenge specific antibody GMT < 32. As in Group 1, LPAI and Indonesian formulations were efficacious (average VE ≥ 48%) to reduce the number of respiratory shedders 2dpc with third order clade viruses; in contrast to Group 1, none of the LPAI formulations were efficacious preventing respiratory viral shedding 2dpc with fourth order clade viruses. The 'unknown' formulation (Indonesian seed of third or fourth order clade per the original article),

had average VE 86% to prevent respiratory shedders in all studies. The efficacy of LPAI, Indonesian and the unknown formulations to prevent death at the end of follow-up was consistent with the VE to prevent respiratory shedding 2dpc. Ducks vaccinated with Pwt06 formulations (v.29, 30) were, in average, less protected against respiratory viral shedding and death compared with the equivalent challenge study in SPF chickens in Group 1 (v.27, 28).

Group 3 gathers studies that performed prime-boosting of two and three doses on SPF, commercial, or native chickens (Fig.S1). Birds were vaccinated between 4 and 16 weeks of age, challenged either 2 or 3wpv, and followed-up to assess survivorship between 7 and 14dpc. Prime-boosting of two doses with LPAI formulations administered to SPF or native chickens resulted in average efficacy ≥ 70% to prevent respiratory viral shedding 2dpc. Despite nuances in respiratory viral shedding protection, these regimens had average efficacy ≥ 95% to prevent death 7 or 10dpc. Only one publication tested prime-boosting of two and three doses using a Pwt06 formulation against a clade homologous challenge (Subang07), finding almost identical protection against death 14dpc.

Group 4 includes studies that performed primary vaccination in 1 or 10-day-old chicks, with and without maternally-derived antibodies (MDA) against AI viruses (Fig.S2). These birds were challenged 3 or 4wpv and followed-up for 10 or 14dpc (Table 1). Among the chicks carrying MDA against Legok03, those vaccinated at one day of age had increased risk of death, while only those vaccinated at 10 days of age with Legok03 were protected against death after the homologous challenge (v.4). In day-old chicks with and without MDA against H5N9, FPvect83 formulations prevented neither respiratory viral shedding 2dpc nor death 4wpv.

Meta-analysis

Meta-analyses were dominated by studies involving LPAI and reversed-genetics formulations. Only formulations carrying Pwt06 were tested across species (Figures 3). I^2 is equal to 0% when the pooled VE is consistent and variation is due to chance; values of 25%, 50%, and 75% approximate low, moderate, and high heterogeneity, attributable to genuine differences of VE being pooled.

Chickens that received a primary dose of LPAI formulations and challenged with a second order clade virus (Indo03) were consistently protected around 50% for viral shedding and 90% for death. Under the same regimen, chickens challenged with third order clade viruses (Smi-Hamd06, Smi-Mae08), were consistently protected against respiratory shedding; however, protection was moderately (I^2= 44.9%) to highly inconsistent (I^2= 82.4%) against cloacal viral shedding and death, respectively. Prime-boosting with LPAI formulations provided consistent protection above 84% against Legok03 in all outcomes measured ($I^2 \leq 38.7\%$).

Against fourth order clade viruses, the pooled VE of primary vaccination with LPAI formulations showed consistent protection against respiratory viral shedding, but less consistent against death after challenge with Papua06 (I^2= 60.2%); against Smi-Pat06, the same regimen had consistent inefficacy to confer any protection while against Pwt06, the pooled VE of prime vaccination was highly inconsistent (I^2 = 95.9% and 73.4%) limiting its interpretability.

The pooled VE of two reversed-genetic formulations against a third (Smi-Hamd06) and fourth (Papua06) order clade viruses revealed consistent protection of chickens. A primary dose of Indonesian formulations carrying Pwt06 seed were tested across species against Suko12; the pooled VE revealed consistent protection of chickens and ducks against respiratory viral shedding 2dpc (I^2= 31.3%), but moderate to highly inconsistent protection against cloacal shedding and death (Figure 3).

Nine subgroups were defined, as described in methods, to explore inconsistency of VE (Figure 4). The pooled subgroup VE of homologous prime-boosting with Mex94 and Eng73 became highly consistent ($I^2 \leq 30.7\%$) against Legok03; likewise, the pooled VE of primary vaccination with Mex94 revealed consistent protection against Smi-Mae08, suggesting that variation in protection was contributed by Wis68 and Eng73 formulations in the global meta-analysis (Figure 5).

The pooled subgroup VE of primary vaccination with Mex94 or Eng73 versus Pwt06 against respiratory shedding remained highly inconsistent ($I^2 = 82.3\%$ and 98.7%), again limiting interpretability; however, the pooled VE of Mex94 formulations against death was moderately inconsistent but low protective, while Eng73 formulations did not protect against death. The subgrouping also confirmed that primary vaccination with Mex94 formulations had no efficacy against Smi-Pat06. Pooled VE of reversed-genetics formulations based on Guang96, did not prevent death in chickens challenged with Pwt06.

The pooled subgroup VE of primary vaccination with Pwt06 formulations against Suko12 revealed that species was a source of VE inconsistency, as the pooled VE of chickens alone had $I^2 = 0\%$ for all outcomes explored (Figure 4).

Discussion

The zoonotic nature and pandemic potential of HPAI/H5N1[1] demands effective vaccine interventions to prevent clinical signs in poultry and significantly reduce viral shedding and onward transmission. The introduction of a new AI clade to Indonesia in 2012 [32] that became dominant in 2016 [33], highlights the importance of continuous evaluation of vaccines. Here, we summarized the performance of commercial monovalent vaccines as Indonesian AI viruses

226 evolved, contributing to a broad body of evidence that emphasizes the importance of regular
227 assessment of vaccines against newly identified AI variants [8, 32, 34-36].

228 The OIE's protocols to assess VE against AI include the use of SPF birds, challenged at least
229 3wpv, using a standardized viral challenge dose able to kill at least 90% of the control group [1].
230 We grouped the studies that most closely resembled the OIE's protocol (Group 1), and formed
231 three other groups that varied such protocol. We found that seroprotective levels induced by
232 vaccines, particularly those induced by LPAI formulations, might not be an accurate indicator of
233 VE against current fourth order clade Indonesian HPAI/H5N1 viruses. According to the OIE's
234 manual [1] efficacious vaccines prevent at least 80% of deaths and induce statistically significant
235 reduction of viral shedding after HPAI/H5N1 challenge. The manual correlates such protection
236 with HI antibody GMT $\geq$ 32 and GMT $\geq$ 128, respectively. We found that vaccine-homologous
237 HI seroprotective levels well above the GMT $\geq$ 128 threshold neither predict protection against
238 respiratory viral shedding 2dpc nor suffice to protect against mortality 14dpc with current fourth
239 order clade viruses. Limitations of this correlation were also evidenced against a second order
240 clade virus challenge (Indo03).

241 Most VE studies are conducted in SPF White Leghorn chickens which tend to have stronger
242 immune response than field birds [7, 29, 37]. Group 2, included studies comparable to those in
243 Group 1 but conducted in commercial chickens. Again, LPAI formulations neither had efficacy
244 preventing respiratory viral shedding 2dpc nor preventing death 14dpc with a fourth order clade
245 virus. These findings in group 1 and 2 are consistent with the poor predictive value attributed to
246 vaccine-homologous HI antibody titers in challenge studies against heterologous AI viruses (8).

247 In comparison, formulations carrying Indonesian seeds conferred adequate protection in both
248 groups, regardless of the antibody titer induced. For instance, Legok03 formulations had average

VE above 83% and 50% against shedding and death across all studies, despite vaccine-homologous HI GMT ≤ 64; furthermore, only vaccines carrying Indonesian seeds were capable to halve the number of birds shedding virus and dying at the end of the study. This suggests that antigenic matching between vaccine-seed and challenge virus is more relevant for adequate protection than the antibody titer. The importance of antigenic matching has been highlighted [36] and demonstrated in previous research [38, 39], while it remains the main reason for the continuous update of human influenza vaccines [40]. Moreover, modern Indonesian experimental vaccines [7], proved to be efficacious against current Indonesian HPAI/H5N1 viruses.

In Group 3, prime-boosting of LPAI formulations conferred equivalent protection against Legok03 in SPF, native, and commercial chickens. This finding contrasts with previous research that suggest limitations of extrapolating VE in SPF to native chickens [41], but this VE may be the result of a better immune response induced by prime-boosting [42]. Homologous prime-boosting of two and three doses of Pwt06 formulations were equally efficacious against Subang07 (clade 2.1.3.2), may be a consequence of comparatively older chickens (18-week-old), that might better cope the viral challenge, and clade-matching of the vaccine seed and challenge virus. Homologous and heterologous prime-boosting have proved successful for AI [42, 43]; however, we only focused on the former as the efficacy of heterologous regimens is beyond the scope of this work. Despite the theoretical benefits of prime-boosting, this strategy is more expensive and logistically complex [44], and it is perceived by some Indonesian producers to pose a mortality risk [45], which might limit its implementation in the field.

A relevant finding in Group 4 was the increased risk of death associated with vaccinating day-old chicks. Early vaccination against AI is practiced in enzootic areas [46] because MDA do not

confer adequate protection [46, 47]; however, when breeder and chicks are vaccinated with the same vaccine seed, these interfere and chicks might be left unprotected [47]. MDA can interfere with vaccination up to 3 weeks post hatching, limiting an early vaccination strategy [46].

Meta-analyses were dominated by LPAI formulations, presumably, due to their widespread long use in Indonesia [48]. LPAI formulations had consistent VE controlling third order clade viruses in chickens, possibly due to the adequate antigenic match between LPAI seeds and clade 2.1.1 [7] and the antigenic similarity of Smi-Mae08 (clade 2.1.3) with clade 2.1.1 [21]. In contrast, this protection was highly inconsistent against fourth order clade viruses as the individual VE ranged from complete (v.8) to nil (v.9) against a single challenge virus. The inconsistent VE remained despite controlling the sources of VE variation in the subgroup meta-analysis. LPAI formulations may be highly immunogenic and may prevent clinical disease regardless of low genetic similarity with the challenge virus [35]; however, we hypothesize that, against fourth order clade viruses, these vaccines at best delay the viral shedding beyond 2dpc, when the virus is expected to cause disease [49]. Our meta-analyses support the decision of limiting vaccination in Indonesia to the use of homologous H5N1 vaccines only [50].

The pooled VE of Pwt06 vaccines against the duck-origin Suko12, showed that extra-label use of vaccines may affect VE consistency. The subgroup meta-analysis showed that VE consistency varied among chickens and ducks which is a clear example of VE as a result of the interaction between the vaccine and the species being vaccinated [48]. Previous studies have shown that inactivated vaccines induce low or undetectable HI antibody titers in ducks [35], but these may still protect against viral challenge [51]; nevertheless, since ducks are now severely affected by HPAI/H5N1 [52], a specific vaccine to protect them and other waterfowls is already available in Indonesia (*Afluvet.* Pusvetma). Overall, the meta-analysis highlighted limitations of LPAI

295 formulations against current fourth order clade viruses, inconsistent VE of alternative seed-
296 homologous vaccines, and drawbacks of extra-label use of vaccines.

297 The ultimate aim of VE assessment is to validate and license vaccine formulations that later will
298 be used under field conditions; however, a limited number of publications have reported the
299 effectiveness of vaccines in Indonesia [45, 53]. One study showed that 90% of poultry would
300 achieve HI antibody GMT $\geq$ 32 after three doses [45]; however, a later study estimated that the
301 effective coverage (i.e. the proportion of the population that would have HI antibody GMT $\geq$ 32),
302 after four quarterly vaccinations would be 34%. Limitations to achieve adequate coverage
303 include resistance of producers to vaccinate [45] and the large number of poultry under different
304 management [53]; furthermore, modelling research has shown that population dynamics of
305 Indonesian sector 4 may undermine the effective coverage of vaccine interventions based on
306 current vaccine technology [54]. These issues represent implementation challenges of translating
307 successful VE assessment into field effectiveness.

308 In conclusion, this review provides evidence that VE prediction based on HI antibody titers alone
309 may not suffice, and that the antigenic relationship between vaccine-seed and challenge virus
310 might be a better indicator of protection. The VE of commercial formulations vary depending on
311 the challenge clade, which highlights the need for ongoing assessment against emergent
312 HPAI/H5N1. The current surveillance platform at the molecular level "IVM Online" [32] has
313 already captured some of these recommendations as new Indonesian HPAI/H5N1 isolates
314 undergo antigenic screening to assess drift and inform vaccine policy. Vaccination has been
315 deemed a driver of antigenic drift [55]; however, evidence to support this hypothesis is lacking in
316 Indonesia [50]. The meta-analyses performed showed that VE consistency is affected by the
317 vaccine formulation, challenge virus and species vaccinated. Finally, since the new dominant

HPAI/H5N1 cause disease in ducks, which are in close contact with native indigenous chickens across Indonesia, further studies to confirm VE in these species are warranted.

Acknowledgements

We thank Ms. Lana Logan for assistance with accurate translation of Indonesian manuscripts into English. We are also grateful of Associate Professor James McCaw for comments that greatly improved the manuscript.

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Tables

Table 1. Characteristics of challenge studies included in the analysis[1]

Publication	Type	Age Vaccination	Vaccine Seed	Vaccine ID (group)	Time Challenge	Challenge Virus	Control
Chickens							
Swayne *et al*. 2006	White Leghorn SPF	3 w	Mex94 Potsdam86	v.12, 26 (group 1)	3wpv	Indo03	Sham Vaccinated (Hepatitis + ND)
Bouma *et al*. 2009	Layer Hens SPF	4 & 7 w	Eng73 Legok03 Mex94	v.6, 7, 16,17, 22, 23 (group 3)	3wpv	Legok03	Unvaccinated

Jadhao *et al*. 2009	White Leghorn SPF	4 w	Mex94	v.13 (group 1)	2wpv	Indo03	Unvaccinated
Poetri *et al*. 2009	Indonesian Native	4 & 7 w	Eng73	v.20 (group 3)	3wpv	Legok03	Unvaccinated
Indriani *et al*. 2011	Layer Hens Isa Brown	3 w	Mex94 Wis68 Eng73 H5N1**	v.14, 15, 21, 24, 44-46 (group 2)	3wpv	Smi-Pat06 Smi-Mae08	Unvaccinated
Poetri *et al*. 2011	Broiler Commercial	1 d 10 d	Legok03	v.3, 4 (group 4)	16dpv 25dpv	Legok03	Unvaccinated
Soejoedono *et al*. 2012	Broiler Commercial	1 d	HVTvect06	v. 40, 41 (group 4)	4wpv	Subang07 Cilingga10	Unvaccinated
Poetri *et al*. 2014	Layer Hens Commercial	4 w	Legok03	v.5 (group 2)	4wpv	Legok03	Unvaccinated
Richard-Mazet *et al*. 2014	Chicken* SPF	1 d	FPvect83	v. 42, 43 (group 4)	4wpv	Subang07	Unvaccinated
Indriani *et al*. 2015	Chicken* SPF	3 w	Pwt06	v.27, 28 (group 1)	3wpv	Suko12	Unvaccinated
Swayne *et al*. 2015	White Leghorn SPF	3 w	Legok03 Mex94 Eng73 Wis68 RG-Guang96 RG-Legok03 RG-Viet04	v.1, 2, 8-11, 18, 19, 25, 34-39 (group 1)	3wpv	Pwt06 Papua06 Smi-Hamd06	Sham Vaccinated (non-infectious allantoic fluid)
Tarigan *et al*. 2015	Layer Hens Commercial	8, 12, 16 w 12 & 16 w 16 w	Pwt06	v.31-33 (group 3)	2wpv	Subang07	Unvaccinated
Ducks							
Indriani *et al*. 2014	Mojosari	3 w	Pwt06	v.29, 30 (group 2)	3wpv	Suko12	Unvaccinated

[1] d= days; w= weeks; dpv= days post-vaccination; wpv= weeks post-vaccination.

Tables

Table 2. Pooled vaccine efficacy (VE), 95% confidence interval (95%CI) and I^2 test against respiratory and cloacal viral shedding 2-day post challenge (dpc) and mortality at the end of follow-up period[1]

Challenge	GMT H	GMT Ch	Oral viral shedding 2dpc	Cloacal viral shedding 2dpc	Mortality end follow-up
LPAI vs Clade 2.1			weight %	weight %	weight %
v.12 Eng73 vs Legok03	128	nr	50	50	50
v.26 Eng73 vs Legok03	120	nr	50	50	50
Pooled VE(95%CI); I^2			43 (15, 61); I^2= 0%	67 (38, 82); I^2= 0%	90 (64, 97); I^2= 0%
LPAI vs Clade 2.1.1 (PB)			weight %	weight %	weight %
v.16 Mex94 vs Legok03	nr	nr	21.10	21.50	20.35
v.17 Mex94 vs Legok03	nr	nr	21.10	21.50	20.35
v.20 Eng73 vs Legok03	891	91	15.60	14.02	18.58
v.22 Eng73 vs Legok03	nr	nr	21.10	21.50	20.35
v.23 Eng73 vs Legok03	nr	nr	21.10	21.50	20.35
Pooled VE (95%CI); I^2			84 (71, 92); I^2= 38.7%	92 (80, 97); I^2= 0%	96 (85, 99); I^2= 0%
LPAI vs Clade 2.1.1			weight %	weight %	weight %
v.8 Mex94 vs Smi-Hamd06	630	nr	33.33	n/a	33.33
v.19 Mex94 vs Smi-Hamd06	169	nr	33.33	n/a	33.33
v.25 Mex94 vs Smi-Hamd06	832	nr	33.33	n/a	33.33
Pooled VE(95%CI); I^2			89 (70, 96); I^2= 0%	n/a	79 (59, 90); I^2= 82.4 %
LPAI vs Clade 2.1.3			weight %	weight %	weight %
v.14 Mex94 vs Smi-Mae08	nr	2.6	25	25	25
v.15 Mex94 vs Smi-Mae08	nr	8.6	25	25	25
v.21 Mex94 vs Smi-Mae08	nr	8.6	25	25	25
v.24 Mex94 vs Smi-Mae08	nr	4.6	25	25	25
Pooled VE(95%CI); I^2			55 (36, 68); I^2= 0%	60 (41, 72); I^2= 44.9 %	76 (59, 86); I^2= 64 %
LPAI vs Clade 2.1.3.1					
v.8 Mex94 vs Papua06	630	nr	33.33	n/a	33.33
v.19 Mex94 vs Papua06	169	nr	33.33	n/a	33.33
v.25 Mex94 vs Papua06	832	nr	33.33	n/a	33.33
Pooled VE(95%CI); I^2			76 (55, 87); I^2= 0%	n/a	70 (48, 82); I^2= 60.2%
LPAI vs Clade 2.1.3.2					
v.14 Mex94 vs Smi-Pat06	nr	1.4	25	25	25
v.15 Mex94 vs Smi-Pat06	nr	8.3	25	25	25
v.21 Eng73 vs Smi-Pat06	nr	1.6	25	25	25
v.24 Wis68 vs Smi-Pat06	nr	0	25	25	25
Pooled VE(95%CI); I^2			14 (0, 26); I^2= 2%	12 (-2, 24); I^2= 38.6%	17 (2, 29); I^2= 31.8%
LPAI vs Clade 2.1.3.2			weight %	weight %	weight %
v.8 Mex94 vs Pwt06	630	0	28.28	n/a	24.70
v.9 Mex94 vs Pwt06	955	0	14.48	n/a	12.65
v.10 Mex94 vs Pwt06	832	0	14.48	n/a	12.65
v.18 Eng73 vs Pwt06	362	0	28.28	n/a	12.65
v.19 Eng73vs Pwt06	169	0	14.48	n/a	24.70
v.11 Mex94 vs Pwt06	294	0	n/a	n/a	12.65
Pooled VE(95%CI); I^2			54 (40, 64); I^2= 95.9 %	n/a	16 (6, 24); I^2= 73.4%
Rev-Gen vs Clade 2.1.1			weight %	weight %	weight %
v.37 Rg-Guang96 vs Smi-Hamd06	73	0	50	n/a	50
v.39 Rg-Viet04 vs Smi-Hamd06	nr	nr	50	n/a	50
Pooled VE(95%CI); I^2			90 (64, 97); I^2= 0%	n/a	95 (68, 99); I^2= 0%
Rev Gen vs Clade 2.1.3.1			weight %	weight %	weight %
v.37 Rg-Guang96 vs Papua06	73	0	50	n/a	50
v.39 Rg-Viet04 vs Papua06	nr	nr	50	n/a	50
Pooled VE(95%CI); I^2			95 (68, 99); I^2= 0%	n/a	81 (54, 92); I^2= 0%
Rev-Gen vs Clade 2.1.3.2			weight %	weight %	weight %
v.34 Rg-Guang96 vs Pwt06	52	0	20	n/a	14.38
v.35 Rg-Guang96 vs Pwt06	34	0	20	n/a	14.38
v.36 Rg-Guang96 vs Pwt06	97	0	20	n/a	14.38
v.38 Rg-Legok03 vs Pwt06	64	nr	20	n/a	14.38
v.39 Rg-Viet04 vs Pwt06	nr	nr	20	n/a	14.38
v.37 Rg-Guang96 vs Pwt06	73	0	n/a	n/a	28.08
Pooled VE(95%CI); I^2			63 (47, 74); I^2= 0%	n/a	21 (9, 30); I^2= 84.6%
Pwt06 vs Clade 2.3.2.1			weight %	weight %	weight %
v.27 Pwt06 vs Suko12	68.6	48.5	27.5	27.5	27.5
v.28 Pwt06 vs Suko12	34.4	17.1	27.5	27.5	27.5
v.29 Pwt06 vs Suko12	10.6	7	22.5	22.5	22.5
v.30 Pwt06 vs Suko12	42.2	18.4	22.5	22.5	22.5
Pooled VE(95%CI); I^2			75 (56, 86); I^2= 31.3%	85 (68, 93); I^2= 75.9%	88 (71, 95); I^2= 60.7%

[1]GMT H: Geometric mean titre against vaccine-homologous antigen derived from hemagglutinin inhibition test; GMT Ch: Geometric mean titre against challenge virus derived from hemagglutinin inhibition test; nr: data not reported; n/a: data not available.

Tables

Table 3. Subgroup meta-analysis. Pooled vaccine efficacy (VE), 95% confidence interval (95%CI) and I^2 test against respiratory and cloacal viral shedding 2-day post challenge (dpc) and mortality at the end of the follow-up period, grouped by type of chicken, species, vaccine seed, and clade of challenge[1]

Challenge	GMT H	GMT Ch	Resp. viral shedding 2dpc	Cloacal viral shedding 2dpc	Mortality end follow-up
(SPF) LPAI vs Clade 2.1.1 (PB)			weight %	weight %	weight %
v.16 Mex94 vs Legok03	nr	nr	50	50	50
v.17 Mex94 vs Legok03	nr	nr	50	50	50
Pooled VE(95%CI); I^2			96 (70, 99); I^2= 0%	96 (70, 99); I^2= 0%	96 (70, 99); I^2= 0%
(SPF) LPAI vs Clade 2.1.1 (PB)			weight %	weight %	weight %
v.22 Eng73 vs Legok03	nr	nr	50	50	50
v.23 Eng73 vs Legok03	nr	nr	50	50	50
Pooled VE (95%CI); I^2			70 (43, 84); I^2= 0%	87 (62, 95); I^2= 30.7%	96 (70, 99); I^2= 0%
(SPF) LPAI vs Clade 2.1.3.2			weight %	weight %	weight %
v.8 Mex94 vs Pwt06	630	0	49.4	n/a	39.42
v.9 Mex94 vs Pwt06	955	0	25.3	n/a	20.19
v.10 Mex94 vs Pwt06	832	0	25.3	n/a	20.19
v.11 Mex94 vs Pwt06	294	0	n/a	n/a	20.19
Pooled VE(95%CI); I^2			67 (49, 79); I^2= 98.7%	n/a	17 (4, 28); I^2= 45.5%
(SPF) LPAI vs Clade 2.1.3.2			weight %	weight %	weight %
v.18 Eng73 vs Pwt06	362	0	66.13	n/a	33.87
v.19 Eng73 vs Pwt06	169	0	33.87	n/a	66.13
Pooled VE(95%CI); I^2			35 (15, 51); I^2= 82.3%	n/a	13 (-2, 26); I^2= 89.9%
(SPF) Rev-Gen vs Clade 2.1.3.2			weight %	weight %	weight %
v.34 Rg-Guang96 vs Pwt06	52	0	33.33	n/a	20.19
v.35 Rg-Guang96 vs Pwt06	34	0	33.33	n/a	20.19
v.36 Rg-Guang96 vs Pwt06	97	0	33.33	n/a	20.19
v.37 Rg-Guang96 vs Pwt06	73	0	n/a	n/a	39.42
Pooled VE(95%CI); I^2			57 (35, 72); I^2= 0%	n/a	12 (0, 22); I^2= 71%
(SPF) Pwt06 vs Clade 2.3.2.1			weight %	weight %	weight %
v.27 Pwt06 vs Suko12	68.6	48.5	50	50	50
v.28 Pwt06 vs Suko12	34.3	17.1	50	50	50
Pooled VE(95%CI); I^2			86 (58, 95); I^2= 0%	95 (67, 99); I^2= 0%	95 (68, 99); I^2= 0%
(Comm) LPAI vs Clade 2.1.3			weight %	weight %	weight %
v.14 Mex94 vs Smi-Mae08	nr	2.6	50	50	50
v.15 Mex94 vs Smi-Mae08	nr	8.6	50	50	50
Pooled VE(95%CI); I^2			52 (24, 70); I^2= 0%	57 (29, 74); I^2= 0%	86 (59, 95); I^2= 31.7%
(Comm) LPAI vs Clade 2.1.3.2			weight %	weight %	weight %
v.14 Mex94 vs Smi-Pat06	nr	1.4	50	50	50
v.15 Mex94 vs Smi-Pat06	nr	8.3	50	50	50
Pooled VE(95%CI); I^2			19 (-3, 37); I^2= 0%	24 (0, 42); I^2= 0%	24 (0, 42); I^2= 0%
(Duck) Pwt06 vs Clade 2.3.2.1			weight %	weight %	weight %
v.29 Pwt06 vs Suko12	10.6	7	50	50	50
v.30 Pwt06 vs Suko12	42.2	18.4	50	50	50
Pooled VE(95%CI); I^2			62 (30, 80); I^2= 0%	73 (42, 88); I^2= 74.7%	78 (48, 91); I^2= 61.5%

[1]GMT H: Hemagglutinin Inhibition geometric mean titre against vaccine-homologous antigen; GMT Ch: Hemagglutinin Inhibition geometric mean titre against challenge virus; SPF: Specific Pathogen Free chicken; Comm: Commercial Chicken; Duck: Mojosari ducks; nr: data not reported; n/a: data not available.

Figures

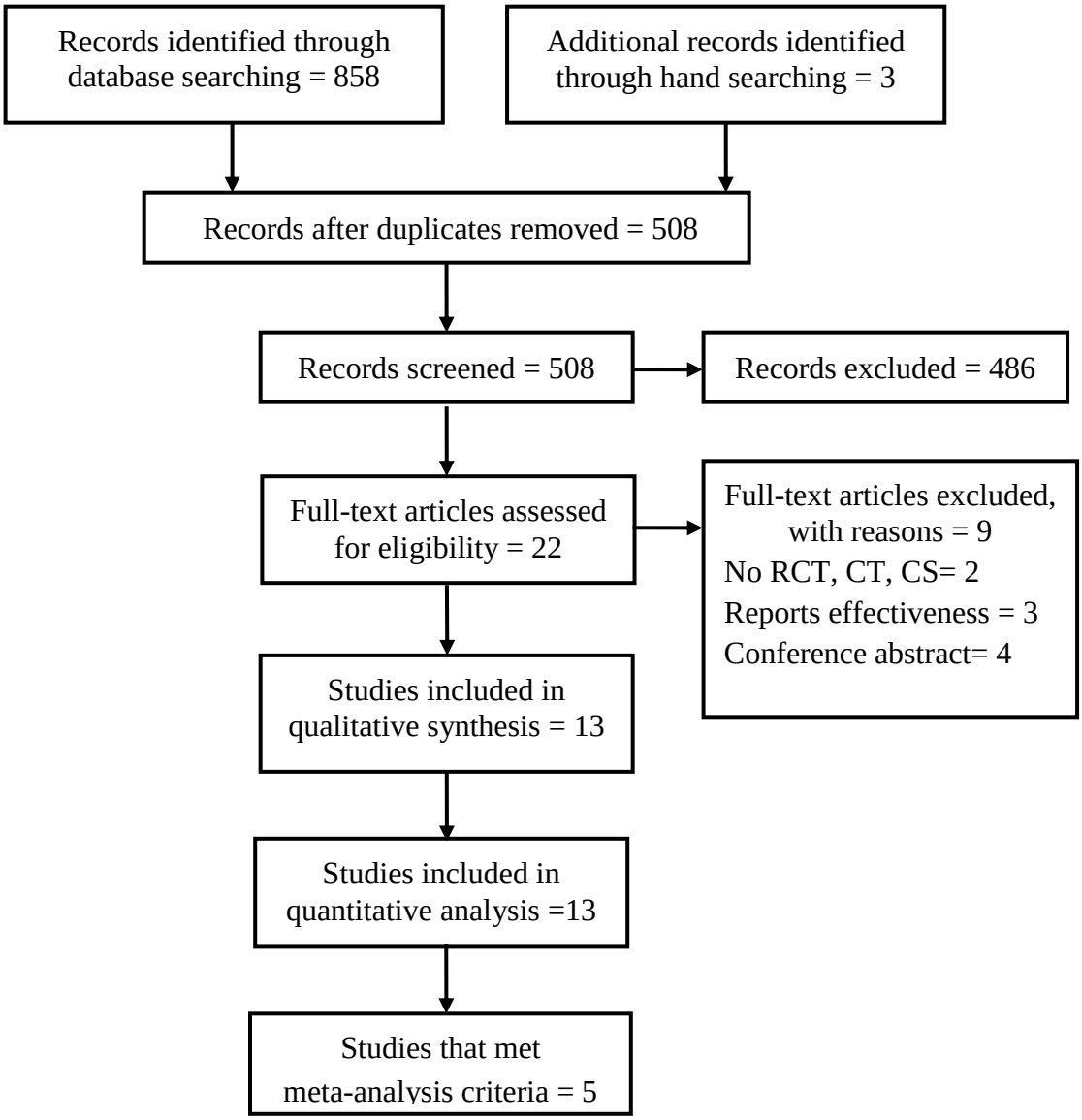

Figure 1. Flow chart of search strategy.

Figures

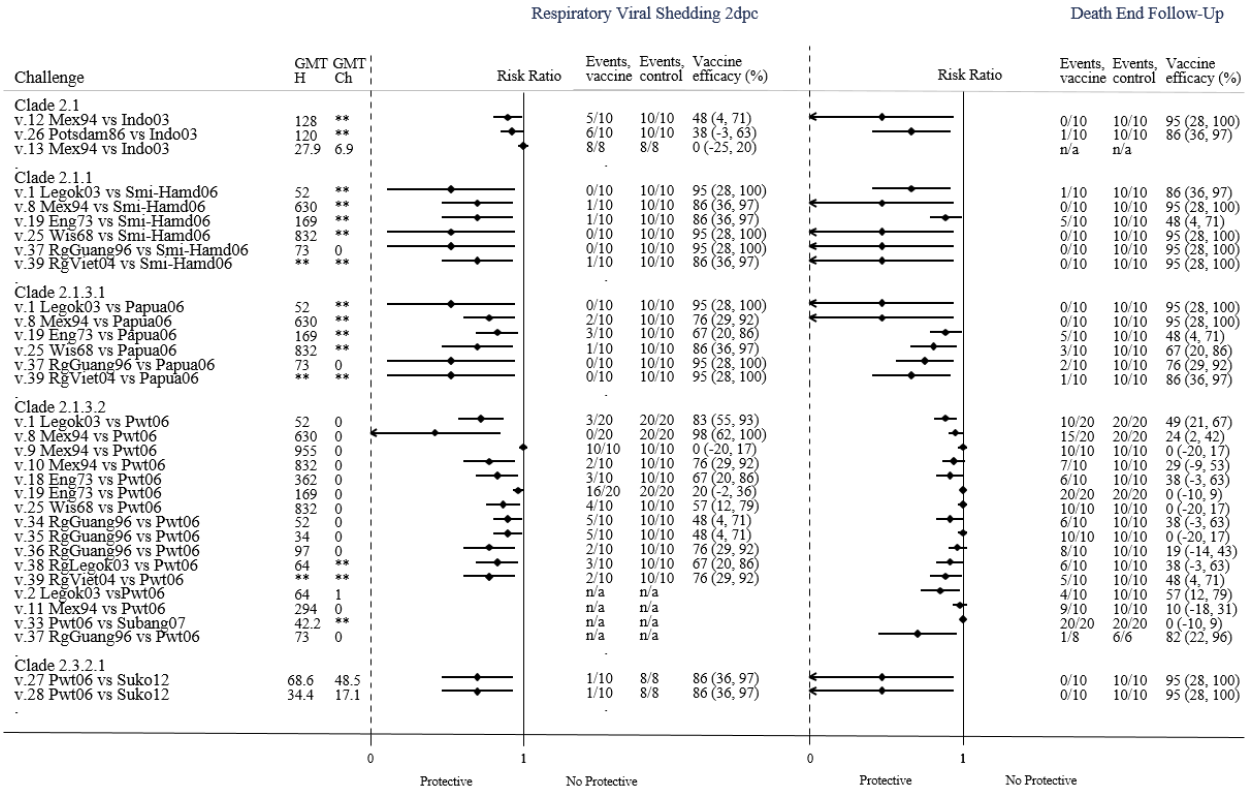

Figure 2. Group 1. Geometric mean titres estimated through hemagglutinin inhibition test against the vaccine-homologous antigen (GMT H) and challenge virus (GMT Ch); risk ratios, vaccine efficacy (1-risk ratio), and corresponding 95% confidence interval against respiratory viral shedding 2-day post challenge (dpc) and death at the end of follow-up period. Asterisks denote GMT not estimated; n/a denote outcome not reported in the original article.

Figures

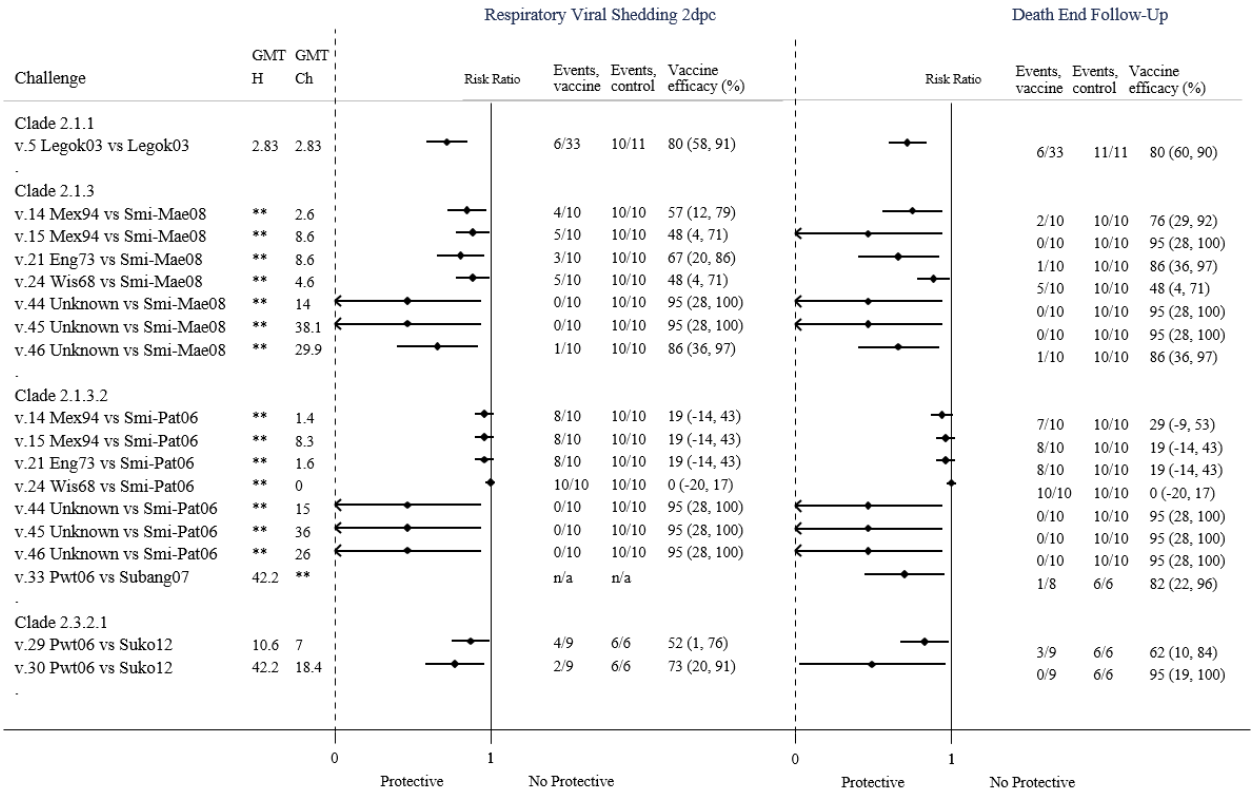

Figure 3. Group 2. Geometric mean titers estimated through hemagglutinin inhibition test against the vaccine-homologous antigen (GMT H) and challenge virus (GMT Ch); risk ratios, vaccine efficacy (1-risk ratio), and corresponding 95% confidence interval against respiratory viral shedding 2-day post challenge (dpc) and death at the end of follow-up period. Asterisks denote GMT not estimated; n/a denote outcome not reported in the original article.

Figures

Table S1. Search terms and number of citations retrieved from Web of Science (core collection). Date of Search: March 17th, 2016

String	Terms	Results
1	(poultry OR chicken* OR duck* OR quail* OR goose OR geese OR turkey* OR *fowl OR broiler* OR layer*)	1,611,843
2	((highly pathogenic avian influenza) OR HPAI OR H5N1 OR avian influenza OR avian flu OR bird flu)	14,260
3	(vaccin* OR immunization OR immunisation OR innocul*)	305,741
4	(Indonesia OR Java OR Borneo OR Sumatra OR Bali)	64,261
5	(disease OR clinical OR subclinical OR infect* OR spread* OR transmi* OR challenge OR sero* OR serum OR antibody)	8,253,725
6	1 AND 2 AND 3 AND 4 AND 5	69

Table S2. Immunogenicity (Heamaglutinin inhibition test's geometric mean titres) and Risk Ratios (RR) for respiratory and cloacal viral shedding, and mortality 2-day post challenge and mortality at the end of follow-up period.

Vaccine			Immunogenicity		Challenge		RR Viral shedding at 2 dpc		RR Mortality			Publication	
ID	**Seed**	**Age vac.**	**Antigen**	**HI Titre**	**N**	**Viral strain**	**Oral route**	**Cloacal route**	**2dpc**	**End study**	**Surv.**	**Ref.**	**Orig. ID**
1	Legok03	3w[a]	Legok03[g] Pwt06[g]	52; 0.3	20	Pwt06[mg]	0.17 (0.07-0.45)[t]	--	0.02 (0.002-0.38)	0.51 (0.33-0.79)[z]	50%	Swayne et al. 2015	K
					10	Papua06 [mg]	0.05 (0.003-0.72) [t]	--	0.05 (0.003-0.72)	0.05 (0.003-0.72)[z]	100%		
					10	Smi-Hamd06[mg]	0.05 (0.003-0.72) [t]	--	0.14 (0.03-0.64)	0.14 (0.03-0.64)[z]	90%		
2	Legok03	3w[a]	Legok03[g] Pwt06 [g]	64; 1	10	Pwt06[mg]	--	--	0.05 (0.003-0.72)	0.43 (0.21-0.88)[z]	60%	Swayne et al. 2015	L
3	Legok03	1d[b] (MDA Legok03)	Legok03[i]	3.25	22	Legok03 [qi]	1 (0.88-1.14) [v] [Oral&Cloaca]	--	1 (0.02-48.2)	1.23 (0.79-1.89)[y]	27%	Poetri et al. 2011	Exp2
4	Legok03	10d[b] (MDA Legok03)	Legok03[k]	3.48	22	Legok03[qk]	0.76 (0.58-1) [v] [Oral&Cloaca]	--	0.33 (0.01-7.76)	0.6 (0.42-0.85)[y]	41%	Poetri et al. 2011	Exp3
5	Legok03	4w[a] (AB)	Legok03 [f]	2.83	33	Legok03[qf]	0.2 (0.1-0.42)[v]	0.11 (0.04-0.29) [v]	--	0.19 (0.09-0.4)[y]	82%	Poetri et al. 2014	Exp
6	Legok03	4&7w[a]	--	--	11	Legok03[qg]	0.04 (0.003-0.66)[v]	0.04 (0.003-0.66)[v]	0.05 (0.003-0.81)	0.04 (0.003-0.66)[x]	100%	Bouma et al. 2009	4
7	Legok03	4&7w[a]	--	--	11	Legok03[qg]	0.04 (0.003-0.66)[v]	0.04 (0.003-0.66)[v]	0.05 (0.003-0.81)	0.04 (0.003-0.66)[x]	100%	Bouma et al. 2009	5
8	Mex94	3w[a]	Mex94[g] Pwt06 [g]	630; 0	20	Pwt06 [mg]	0.02 (0.002-0.38) [t]	--	0.02 (0.002-0.38)	0.76 (0.58-0.98)[z]	25%	Swayne et al. 2015	E
					10	Papua06[mg]	0.24 (0.08-0.71) [t]	--	0.05 (0.003-0.72)	0.05 (0.003-0.72)[z]	100%		
					10	Smi-Hamd06[mg]	0.14 (0.03-0.64) [t]	--	0.05 (0.003-0.72)	0.05 (0.003-0.72)[z]	100%		
9	Mex94	3w[a]	Mex94 [g] Pwt06 [g]	955; 0	10	Pwt06[mg]	1 (0.83-1.2) [t]	--	0.91 (0.69-1.18)	1 (0.83-1.2)[z]	0%	Swayne et al. 2015	I
10	Mex94	3w[a]	Mex94 [g] Pwt06 [g]	832; 0	10	Pwt06[mg]	0.24 (0.08-0.71) [t]	--	0.24 (0.08-0.71)	0.71 (0.47-1.09)[z]	30%	Swayne et al. 2015	J
11	Mex94	3w[a]	Mex94 [g] Pwt06 [g]	294; 0.1	10	Pwt06[mg]	--	--	0.43 (0.21-0.88)	0.91 (0.69-1.18)[z]	10%	Swayne et al. 2015	M
12	Mex94	3w[a]	Mex94 [g]	128	10	Indo03[mg]	0.5 (0.29-0.96)[v]	0.33 (0.14-0.8)[v]	--	0.05 (0.003-0.72)[z]	100%	Swayne et al. 2006	Nobilis I.A.
13	Mex94	4w[c]	Mex94[h]; Indo03[h]	27.9; 6.9	8	Indo03[nh]	1 (0.8-1.25) [v]	--	0.06 (0.004-0.87)	--	--	Jadhao et al. 2009	Nobilis
14	Mex94	3w[a]	Smi-Pat06[g]; Smi-Mae08[g]	1.4; 2.6	10	Smi-Pat06[og]	0.81 (0.57-1.14)[v]	0.71 (0.47-1.09)[v]	--	0.71 (0.47-1.09)[z]	30%	Indirani et al. 2011	F

					10	Smi-Mae08[og]	0.43 (0.21-0.88)[v]	0.52 (0.29-0.96)[v]	--	0.24 (0.08-0.71)[z]	80%		
15	Mex94	3w[a]	Smi-Pat06[g]; Smi-Mae08[g]	8.3; 8.6	10	Smi-Pat06[og]	0.81 (0.57-1.14)[v]	0.81 (0.57-1.14)[v]	--	0.81 (0.57-1.14)[z]	20%	Indirani et al. 2011	D
					10	Smi-Mae08[og]	0.52 (0.29-0.96)[v]	0.33 (0.14-0.80)[v]	--	0.05 (0.003-0.72)[z]	100%		
16	Mex94	4 & 7w[a]	--	--	11	Legok03[sg]	0.04 (0.003-0.66)[v]	0.04 (0.003-0.66)[v]	1 (0.02-46.4)	0.04 (0.003-0.66)[x]	100%	Bouma et al. 2009	3
17	Mex94	4 & 7w[a]	--	--	11	Legok03[qg]	0.04 (0.003-0.66)[v]	0.04 (0.003-0.66)[v]	0.05 (0.003-0.81)	0.04 (0.003-0.66)[x]	100%	Bouma et al. 2009	6
18	Eng73	3w[a]	Eng73[g] Pwt06[g]	362; 0	10	Pwt06[mg]	0.33 (0.14-0.8)[t]	--	0.05 (0.003-0.72)	0.62 (0.37-1.03)[z]	40%	Swayne et al. 2015	A
19	Eng73	3w[a]	Eng73[g] Pwt06[g]	169; 0	20	Pwt06[mg]	0.81 (0.64-1.02)[t]	--	0.22 (0.09-0.49)	1 (0.91-1.1)[z]	0%	Swayne et al. 2015	B
					10	Papua06[mg]	0.33 (0.14-0.8)[t]	--	0.24 (0.08-0.72)	0.52 (0.29-0.96)[z]	50%		
					10	Smi-Hamd06[mg]	0.14 (0.03-0.64)[t]	--	0.24 (0.08-0.72)	0.52 (0.29-0.96)[z]	50%		
20	Eng73	4 & 7w[b]	Eng73[g]; Legok03[g]	891; 91	10	Legok03[pg]	0.06 (0.004-0.9)[v]	0.07 (0.004-1.03)[v]	0.07 (0.004-0.99)	0.05 (0.003-0.72)[y]	100%	Poetri et al. 2009	A Vaksiflu N2 PT
21	Eng73	3w[a]	Smi-Pat06[g]; Smi-Mae08[g]	1.6; 8.6	10	Smi-Pat06[og]	0.81 (0.57-1.14)[v]	1 (0.83-1.2)[v]	--	0.81 (0.57-1.14)[z]	20%	Indirani et al. 2011	G
						Smi-Mae08[og]	0.33 (0.14-0.8)[v]	0.14 (0.03-0.64)[v]	--	0.14 (0.03-0.64)[z]	90%		
22	Eng73	4 & 7w[a]	--	--	11	Legok03[sg]	0.3 (0.13-0.74)[v]	0.04 (0.003-0.66)[v]	1 (0.02-46.4)	0.04 (0.003-0.66)[x]	100%	Bouma et al. 2009	1
23	Eng73	4 & 7w[a]	--	--	11	Legok03[sg]	0.3 (0.13-0.74)[v]	0.22 (0.07-0.66)[v]	1 (0.02-46.4)	0.04 (0.003-0.66)[x]	100%	Bouma et al. 2009	2
24	Wis68	3w[a]	Smi-Pat06[g]; Smi-Mae08[g]	0; 4.6	10	Smi-Pat06[og]	1 (0.83-1.2)[v]	1 (0.83-1.2)[v]	--	1 (0.83-1.2)[z]	0%	Indirani et al. 2011	E
					10	Smi-Mae08[og]	0.52 (0.29-0.96)[v]	0.62 (0.37-1.03)[v]	--	0.52 (0.29-0.96)[z]	50%		
25	Wis68	3w[a]	Wis68[g]; Pwt06[g]	832; 0	10	Pwt06[mg]	0.43 (0.21-0.88)[t]	--	0.05 (0.003-0.72)	1 (0.83-1.2)[z]	0%	Swayne et al. 2015	F
					10	Papua06[mg]	0.14 (0.03-0.64)[t]	--	0.14 (0.03-0.64)	0.33 (0.14-0.79)[z]	70%		
					10	Smi-Hamd06[mg]	0.05 (0.003-0.72)[t]	--	0.05 (0.003-0.72)	0.05 (0.003-0.72)[z]	100%		

26	Potsdam86	3w[a]	Posdam86[g]	120	10	Indo03[mg]	0.62 (0.37-1.03)[v]	0.33 (0.14-0.8)[v]	--	0.14 (0.03-0.64)[z]	90%	Swayne et al. 2006	EXP-Nobilis
27	Pwt06	3w[a]	Pwt06[g]; Suko12[g]	68.6; 48.5	10	Suko12[og]	0.14 (0.03-0.64)[v]	0.05 (0.003-0.73)[v]	--	0.05 (0.003-0.72)[z]	100%	Indriani et al. 2015	A
28	Pwt06	3w[a]	Pwt06[g]; Suko12[g]	34.3; 17.1	10	Suko12[og]	0.14 (0.03-0.64)[v]	0.05 (0.003-0.73)[v]	--	0.05 (0.003-0.72)[z]	100%	Indriani et al. 2015	B
29	Pwt06	3w[a] (ducks)	Pwt06[g]; Suko12[g]	10.6; 7	9	Suko12[og]	0.49 (0.24-0.99)[v]	0.49 (0.24-0.99)[v]	--	0.37 (0.16-0.86)[z]	67%	Indriani et al. 2014	A
30	Pwt06	3w[a] (ducks)	Pwt06[g]; Suko12[g]	42.2; 18.4	9	Suko12[og]	0.27 (0.09-0.8)[v]	0.05 (0.004-0.81)[v]	--	0.05 (0.004-0.78)[z]	100%	Indriani et al. 2014	B
31	Pwt06	8, 12 & 16w[a]	Pwt06[h]	97	8	Subang07[rh]	--	--	--	0.29 (0.10-0.88)[z]	75%	Tarigan et al. 2015	Medivac AI
32	Pwt06	12 & 16w[a]	Pwt06[h]	84.4	8	Subang07[rh]	--	--	--	0.18 (0.04-0.79)[z]	88%	Tarigan et al. 2015	Medivac AI
33	Pwt06	16w[a]	Pwt06[h]	42.2	8	Subang07[rh]	--	--	--	0.18 (0.04-0.79)[z]	88%	Tarigan et al. 2015	Medivac AI
34	Rg Guang96	3w[a]	Guang96[g] Pwt06[g]	52; 0	10	Pwt06[mg]	0.52 (0.29-0.96)[t]	--	0.14 (0.03-0.64)	0.62 (0.37-1.03)[z]	40%	Swayne et al. 2015	D
35	Rg Guang96	3w[a]	Guang96[g] Pwt06[g]	34; 0	10	Pwt06[mg]	0.52 (0.29-0.96)[t]	--	0.52 (0.29-0.96)	1 (0.83-1.2)[z]	0%	Swayne et al. 2015	G
36	Rg Guang96	3w[a]	Guang96[g] Pwt06[g]	97; 0	10	Pwt06[mg]	0.24 (0.08-0.71)[t]	--	0.05 (0.003-0.72)	0.81 (0.57-1.14)[z]	20%	Swayne et al. 2015	H
37	Rg Guang96	3w[a]	Guang96[g] Pwt06[g]	73; 0	20	Pwt06[mg]	--	--	0.32 (0.17-0.60)	1 (0.91-1.1)[z]	0%	Swayne et al. 2015	N
					10	Papua06[mg]	0.05 (0.003-0.72)[t]	--	0.05 (0.003-0.72)	0.24 (0.08-0.71)[z]	80%		
					10	Smi-Hamd06[mg]	0.05 (0.003-0.72)[t]	--	0.05 (0.003-0.72)	0.05 (0.003-0.72)[z]	100%		
38	Rg Legok03	3w[a]	Legok03[g]	64	10	Pwt06[mg]	0.33 (0.14-0.8)[t]	--	0.05 (0.003-0.72)	0.62 (0.37-1.03)[z]	40%	Swayne et al. 2015	C
39	Rg Viet04		--	--	10	Pwt06[mg]	0.24 (0.08-0.71)[t]	--	0.14 (0.03-0.64)	0.52 (0.29-0.96)[z]	50%	Swayne et al. 2015	O
					10	Papua06[mg]	0.05 (0.003-0.72)[t]	--	0.05 (0.003-0.72)	0.14 (0.03-0.64)[z]	90%		
					10	Smi-Hamd06[mg]	0.14 (0.03-0.64)[t]	--	0.05 (0.003-0.72)	0.05 (0.003-0.72)[z]	100%		
40	HV vect06	1d[a] (MDA H5N1)	Egypt06[f]; Nagrak07[f]; Subang07[f]	141; 8.3; 3.8	ND	Subang07[mf]	--	--	NA	NA[z]	80%	Soejoedno et al. 2012	Vectormune HVT t1
41	HV vect06	1d[a] (MDA H5N1)	Egypt06[f]; Nagrak07 & B-Tang10[f]; WJ-PC10[f]	24.4; 4.4; 3.6	ND	Cilingga10[mf]	--	--	NA	NA[z]	95%	Soejoedno et al. 2012	Vectormune HVT t2

42	FP vect83	1d[d]	Ireland83[f]; Italy98[f]; Subang07[f]	45.3; 7.5; < 8	10	Subang07[mf]	1 (0.83-1.2)[t]	--	0.05 (0.003-0.72)	0.91 (0.69-1.18)[z]	10%	Richard-Mazet et al. 2014	Trovac AIV H5
43	FP vect83	1d[d] (MDA H5N9)	Ireland83[f]; Italy98[f]; Subang07[f]	7.5; 4; < 8	9	Subang07[mf]	1 (0.82-1.21)[t]	--	0.05 (0.004-0.79)	1 (0.82-1.22)[z]	0%	Richard-Mazet et al. 2014	Trovac AIV H5
44	unknown (H5N1)*	3w[a]	Smi-Pat06[g]; Smi-Mae06[g]	15; 14	10	Smi-Pat06[og]	0.05 (0.003-0.72)[t]	0.05 (0.003-0.72)[t]	--	0.05 (0.003-0.72)[z]	100%	Indirani et al. 2011	A
					10	Smi-Mae08[og]	0.05 (0.003-0.72)[t]	0.05 (0.003-0.72)[t]	--	0.05 (0.003-0.72)[z]	100%		
45	unknown (H5N1)*	3w[a]	Smi-Pat06[g]; Smi-Mae08[g]	36; 38.1	10	Smi-Pat06[og]	0.05 (0.003-0.72)[t]	0.05 (0.003-0.72)[t]	--	0.05 (0.003-0.72)[z]	100%	Indirani et al. 2011	B
					10	Smi-Mae08[og]	0.05 (0.003-0.72)[t]	0.05 (0.003-0.72)[t]	--	0.05 (0.003-0.72)[z]	100%		
46	unknown (H5N1)*	3w[a]	Smi-Pat06[g]; Smi-Mae08[g]	26; 29.9	10	Smi-Pat06[og]	0.05 (0.003-0.72)[t]	0.05 (0.003-0.72)[t]	--	0.05 (0.003-0.72)[z]	100%	Indirani et al. 2011	C
					10	Smi-Mae08[og]	0.14 (0.03-0.64)[t]	0.14 (0.03-0.64)[t]	--	0.14 (0.03-0.64)[z]	90%		

Dose vaccination: [a] Per Product Information; [b] 256HAU; [c] 0.5mL; [d] 3log10TCID50; [e] 0.0125ugHA.
Time Immunogenicity: [f]= 4wpv; [g]=3wpv; h=2wpv; [i] =25dpv; [k]=16dpv. (wpv= week-post vaccination; dpv= day-post vaccination).
Time and dose Viral challenge: [m]= 10^6 EID50 / IN; [n]= 10^6 EID50 / ON; [o] = 0.1mL 10^6 EID50 / IN; [p]= 0.1mL 10^6 EID50 / IT; [q]= 0.2mL 10^6 EID50 / IN,IT; [r]= 10^5 EID50 / OP; [s]= 0.2mL 10^5 EID50 / IN,IT; (IN=Intra-nasal; ON=oro-nasal; IT= Intra-tracheal; OP= Oropharyngeal).
Follow-up period post challenge: [X] =7days; [Y] = 10 days; [Z] =14 days
Viral identification method: [t]=PCR; [v]=egg isolation or egg titration.
ND= number of birds not declared. *Seed not identified.

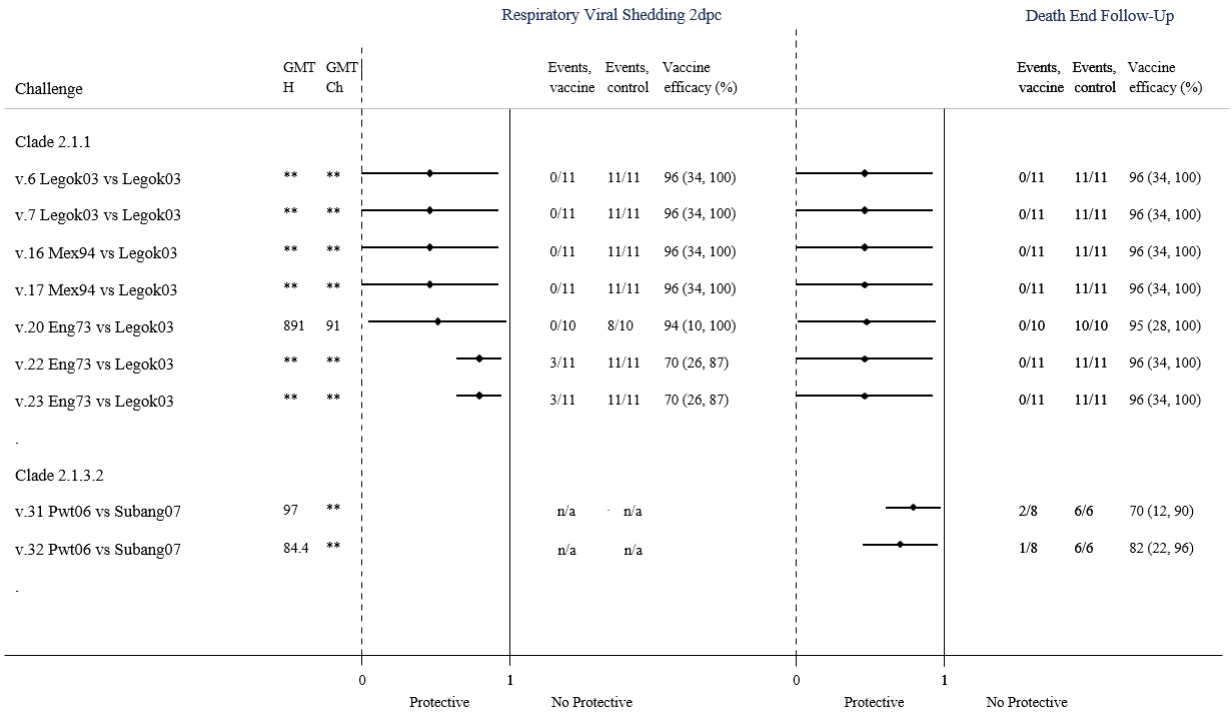

Figure S1. Group 3. Geometric mean titres estimated through hemagglutinin inhibition test against the vaccine-homologous antigen (GMT H) and challenge virus (GMT Ch); risk ratios, vaccine efficacy (1- risk ratio), and corresponding 95% confidence interval against respiratory viral shedding 2-day post challenge (dpc) and death at the end of follow-up period. Asterisks denote GMT not estimated; n/a denote outcome not reported in the original article.

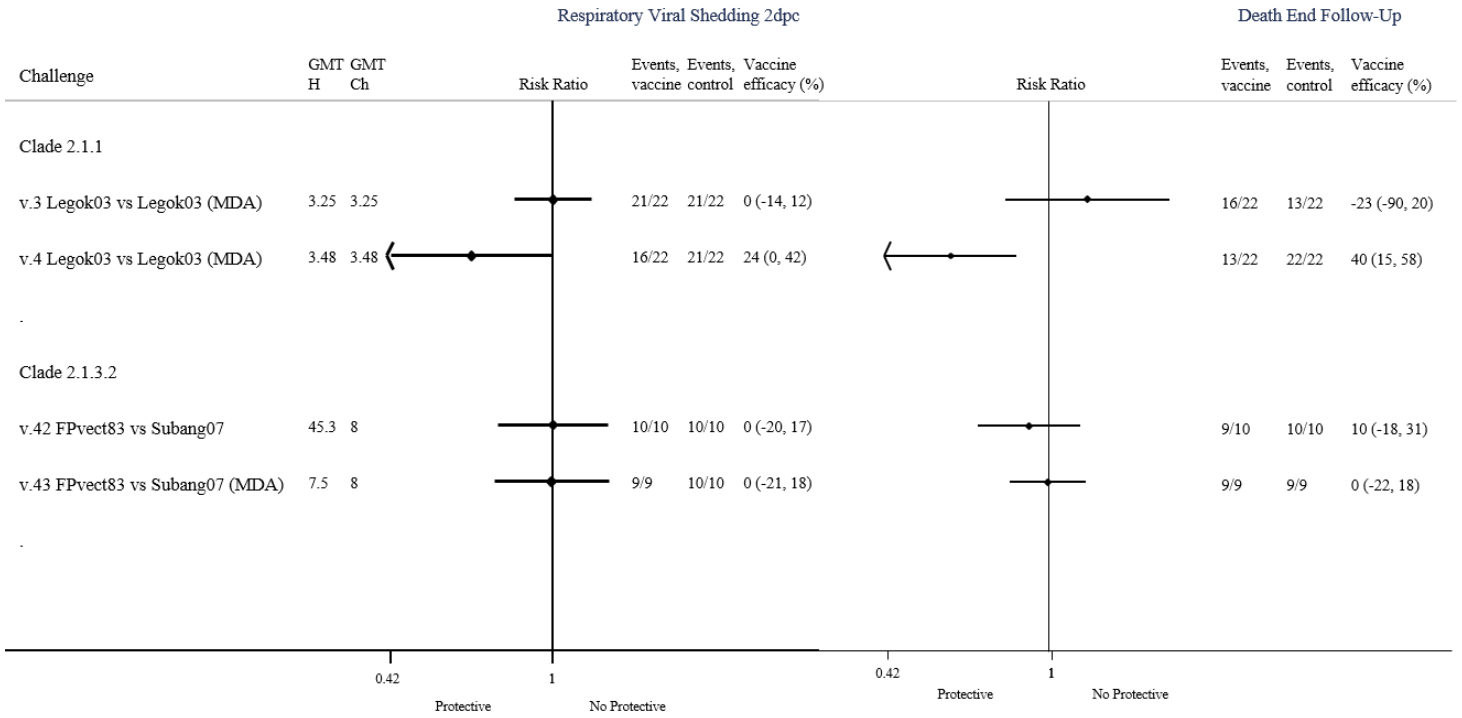

Figure S2. Group 4. Geometric mean titres estimated through hemagglutinin inhibition test against the vaccine-homologous antigen (GMT H) and challenge virus (GMT Ch); risk ratios, vaccine efficacy (1- risk ratio), and corresponding 95% confidence interval against respiratory viral shedding 2-day post challenge (dpc) and death at the end of follow-up period. Asterisks denote GMT not estimated; n/a denote outcome not reported in the original article.

Table S3. Risk of Bias assessment

Publication	***a***	***b***	***c***	***d***	***e***	***f***	***g***
Swayne *et al.*, 2006	**?**	**?**	+	+	+	+	+
Jadhao *et al.*, 2009	**?**	-	+	+	-	+	+
Bouma *et al.*, 2009	**?**	**?**	+	+	+	+	+
Poetri *et al.*, 2009	**?**	**?**	+	+	+	+	+
Poetri *et al.*, 2011	**?**	**?**	+	+	+	-	+
Indriani *et al.*, 2011	**?**	**?**	+	+	+	+	**?**
Soejoedno *et al.*, 2012	**?**	**?**	+	+	+	-	**?**
Richard-Mazet *et al.*, 2014	**?**	-	+	+	+	+	+
Poetri *et al.*, 2014	**?**	-	+	+	+	+	-
Indriani *et al.*, 2014	**?**	**?**	+	+	+	+	+
Swayne *et al.*, 2015	**?**	**?**	+	+	+	+	+
Tarigan *et al.*, 2015	**?**	**?**	+	+	+	-	+
Indriani *et al.*, 2015	**?**	**?**	+	+	+	+	+

a= Random sequence generation (Selection Bias); b= Allocation concealment (Selection Bias); c= Blinding of researchers (Performance Bias); d= Blinding of outcome assessment (Detection Bias); e= Incomplete outcome data (Attrition Bias); f= Selective Reporting (Reporting Bias); g= Other bias.

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Author/s:
Villanueva-Cabezas, JP; Coppo, MJC; Durr, PA; McVernon, J

Title:
Vaccine efficacy against Indonesian Highly Pathogenic Avian Influenza H5N1: systematic review and meta-analysis

Date:
2017-09-05

Citation:
Villanueva-Cabezas, J. P., Coppo, M. J. C., Durr, P. A. & McVernon, J. (2017). Vaccine efficacy against Indonesian Highly Pathogenic Avian Influenza H5N1: systematic review and meta-analysis. VACCINE, 35 (37), pp.4859-4869. https://doi.org/10.1016/j.vaccine.2017.07.059.

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