always occur concurrently was made in the underlying model of spread. This remained true even when interventions such as vaccination [8] were included in the forward projections (Figure 1E). (For further information about our analysis and additional discussion, see the Supplementary Material.)

For forecasting the effects of some interventions, such as those that reduce the time between symptom onset and isolation, it might be necessary to ensure that infectious periods and symptomatic periods are accurately represented by the epidemiological model. However, for predicting the impacts of vaccination campaigns during Ebola outbreaks, it may not be necessary to measure the lengths of these periods with absolute precision. Careful testing of model assumptions during outbreaks—as well as long-term engagement between clinicians, modelers, and policy makers—will help optimize the development of public health policy.

SUPPLEMENTARY DATA

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Role of Egg-adaptation Mutations in Low Influenza A(H3N2) Vaccine Effectiveness During the 2012–2013 Season

To THE EDITOR—The egg-adapted A(H3N2) vaccine component IVR-165 was associated with low influenza vaccine effectiveness (VE approximately 40%) during the 2012–2013 season [1]. IVR-165 bore 3 amino acid substitutions (H156Q, G186V, and S219Y) compared to the vaccine strain recommended by the World Health Organization (A/Victoria/361/2011). Notably, position 156 is located near the receptor binding site within immunodominant antigenic site B at the top of the hemagglutinin head and is 1 of just 7 positions associated with all major A(H3N2) antigenic cluster transitions since 1968 [2]. As such, the in vitro H156Q reversion that occurred with egg adaptation of the 2012–2013 vaccine strain is thought to have contributed to low influenza VE that season [1].

In their recent publication, Cobey et al hypothesize that vaccine mismatch due to egg-adaptation mutations should be evident as a different profile of influenza variants infecting vaccinated compared to unvaccinated people, whereas their sequence analysis detected no difference in 2012–2013 [3]. Their hypothesis, however, does not seem valid. By way of illustration, Cobey and co-authors have also proposed that egg-adaptation mutations (notably T160K, a loss of glycosylation) [4] played a key role in the low VE (<40%) against A(H3N2) in 2016–2017 and 2017–2018 [5–8]. However, they did not test their hypothesis of differing influenza variants by vaccine status for those particular seasons. In fact, viruses sequenced from Canadian VE study participants showed that there were also no differences in the profile of infecting influenza variants by vaccination status in 2016–2017 (n = 574) or 2017–2018 mid-season (n = 229; Table 1). We do not interpret those findings as ruling out a role for egg-adaptation mutations. Instead, and contrary to the assumption of Cobey et al, if egg-adaptation mutations affect antigenicity and reduce the immunogenicity of seasonal vaccine, then the infecting A(H3N2) strain should be independent of vaccination status—as observed in our data for 2016–2018 and also by Cobey et al for 2012–2013.

Cobey et al further argue that, unlike anti-sera drawn from naive ferrets, anti-sera collected from adults vaccinated with the egg-adapted IVR-165 do not distinguish it from the recommended vaccine strain or circulating clade 3C.2 or 3C.3 viruses [3]. They report titers pre-vaccination and fold changes post-vaccination that were highly correlated across these test viruses. However, their correlations were driven by a majority of titers that started low and showed minimal or no vaccine-induced

change. Their serologic analyses pooled just 28 adults aged 30–40 years and 33 adults aged 65–87 years. Although their figure 2 does not permit exact quantification owing to overlapping pairs and missing data, most sera displayed a <4-fold rise in vaccine-induced titers and a substantial proportion showed a <2-fold rise (within the margin of error of the dilutional hemagglutination inhibition assay) [9]. Although 20/56 (36%) participants seroconverted to IVR-165, this pooled finding is also difficult to interpret in the context of conventional immunogenicity thresholds for annual vaccine approval requiring seroconversion in at least 40% of young adults and 30% of elderly adults [10]. Either way, without the comparator of sera drawn from adults vaccinated with cell culture–based (or other non-egg–based) vaccine, the serologic findings presented by Cobey et al do not resolve a role for egg-adaptation mutations.

Ultimately, egg-adaptation mutations that result in altered antigenicity and poor immunological responses (including minimal boosting of cross-reactive antibody) are not mutually exclusive phenomena. As we have underscored previously, more definitive investigations are needed to understand how these alterations may interact with other agent-host factors to modulate VE, including variation in priming epochs, birth cohort effects, and underlying immunological landscapes [11].

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Table 1. Clade Distribution of A(H3N2) Viruses by Vaccination Status, Canadian Sentinel Practitioner Surveillance Network

Clade/Variant[a]	Unvaccinated, n (%)	Vaccinated,[b] n (%)	*P* Value[c]
A. 2016–2017 full season analysis (1 November 2016 to 30 April 2017)[d]			
Clade 3C.2a	100 (23)	30 (22)	.85
+ N31S + D53N + R142G + S144R + N171K + I192T + Q197H	1 (0)	0 (0)	1.00[e]
+ N121K + S144K	31 (7)	10 (7)	.91
+ T131K + R142K + R261Q	61 (14)	20 (15)	.82
Other substitutions	7 (2)	0 (0)	.21[e]
Clade 3C.2a1	320 (73)	103 (76)	.54
Other substitutions without N121K	28 (6)	10 (7)	.69
+ N121K + K92R + H311Q	61 (14)	12 (9)	.12
+ N121K + R142G	66 (15)	26 (19)	.26
+ N121K + T135K + HA2:G150E	67 (15)	26 (19)	.29
+ N121K + I140M + HA2:G150E	3 (1)	4 (3)	.06[e]
+ N121K + R142G + I242V + HA2:G150E	89 (20)	24 (18)	.49
N121K + other substitutions	6 (1)	1 (1)	1.00[e]
Clade 3C.3a	18 (4)	3 (2)	.43[e]
Total	**438 (100)**	**136 (100)**	
B. 2017–2018 mid-season analysis (5 November 2017 to 13 January 2018)[f]			
Clade 3C.2a	142 (93)	71 (93)	.86
+ N31S + D53N + R142G + S144R + N171K + I192T + Q197H	3 (2)	0 (0)	.55[e]
+ N121K + S144K	4 (3)	2 (3)	1.00[e]
+ T131K + R142K + R261Q	135 (88)	69 (91)	.56
Clade 3C.2a1	11 (7)	4 (5)	.78[e]
+ N121K + K92R + H311Q	9 (6)	3 (4)	.76[e]
+ N121K + T135K + HA2:G150E	2 (1)	1 (1)	1.00[e]
Clade 3C.3a	0 (0)	1 (1)	.33[e]
Total	**153 (100)**	**76 (100)**	

[a]Specimens were tested for influenza viruses using reverse-transcriptase polymerase chain reaction at provincial public health reference laboratories as previously described [5, 7]. Genetic characterization of the hemagglutinin was attempted on all influenza-positive original specimens collected from Canadian Sentinel Practitioner Surveillance Network patients using Sanger sequencing. Phylogenetic analysis was conducted based on nucleotide sequence using the approximate likelihood method to determine clade distribution and identify major genetic clusters (or "parent" groups) in conjunction with published reports. See Supplementary Materials for more details and references related to sequence analysis.

[b]Vaccination status ascertained as per usual based on patient self-report and sentinel practitioner documentation. Patients who self-reported receipt of ≥1 dose of the current season's influenza vaccine ≥2 weeks before onset of influenza-like illness (ILI) were considered vaccinated; those vaccinated <2 weeks before ILI onset were excluded.

[c]*P* values based on χ^2 test comparing the proportion of viruses within the specified clade/variant vs all other clades/variants among vaccinated vs unvaccinated participants.

[d]Methods as per [5], but including viruses with specimen collection dates spanning up to 30 April 2017. Associated GenBank sequence numbers for 564 of 574 included viruses are KY583507 to KY583727, MH216203–MH216328, MH216331–MH216445, and MH216447–MH216548. Ten sequences were of insufficient quality for GenBank submission but were sufficient for clade/variant determination based on clade-defining amino acid substitutions.

[e]Fisher's exact test used where >25% of expected cell counts were <5.

[f]Methods as per [7]. Associated GenBank sequence numbers of included viruses are MG889597– MG889825.

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Response to Skowronski and De Serres

To the Editor—Skowronski and De Serres make several claims that warrant clarification. In our study, we sought to explain the low effectiveness of the influenza A/H3N2 vaccine in 2012–2013. We first examined whether the influenza virus variants that infected vaccinated and unvaccinated people differed [1]. We did not find a difference, suggesting that antigenic variation in a subset of viruses was not responsible for low vaccine effectiveness. However, this phylogenetic test for antigenic mismatch is imperfect. While a clear difference would be meaningful, the lack of a difference could be attributable to several alternatives, including swamping of the signal by poor immunogenicity. Therefore, we then examined other evidence, using human, rather than ferret, serological data to evaluate the response to the intended vaccine strain (A/Victoria/361/2011), the egg-adapted vaccine strain (IVR-165), and strains from the 2 dominant circulating clades. We note that previously Skowronski and colleagues used data derived from ferret experiments to postulate that the egg-adaptation mutations in IVR-165 lowered vaccine effectiveness by causing antigenic mismatch with the circulating strains [2]. Our results underscore that the vaccine was poorly immunogenic, as Skowrosonski and de Serres note in their letter, and also demonstrate that the patterns of reactivity in humans are nonetheless inconsistent with ferret-based inferences. The use of samples from 61 human donors is, to our knowledge, the largest published analysis of human serological responses directed against egg vs non-egg H3N2 viruses from this season and highlights how ferret data alone can mislead. We agree with Skowronski and De Serres that altered antigenicity and poor immunological responses are not mutually exclusive and that comprehensive studies to evaluate the factors that contribute to the modest effectiveness of influenza vaccines are desperately needed.

Notes

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