

# Emergenet: Fast Scalable Pandemic Risk Estimation of Influenza A Strains Collected In Non-human Hosts

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## Abstract

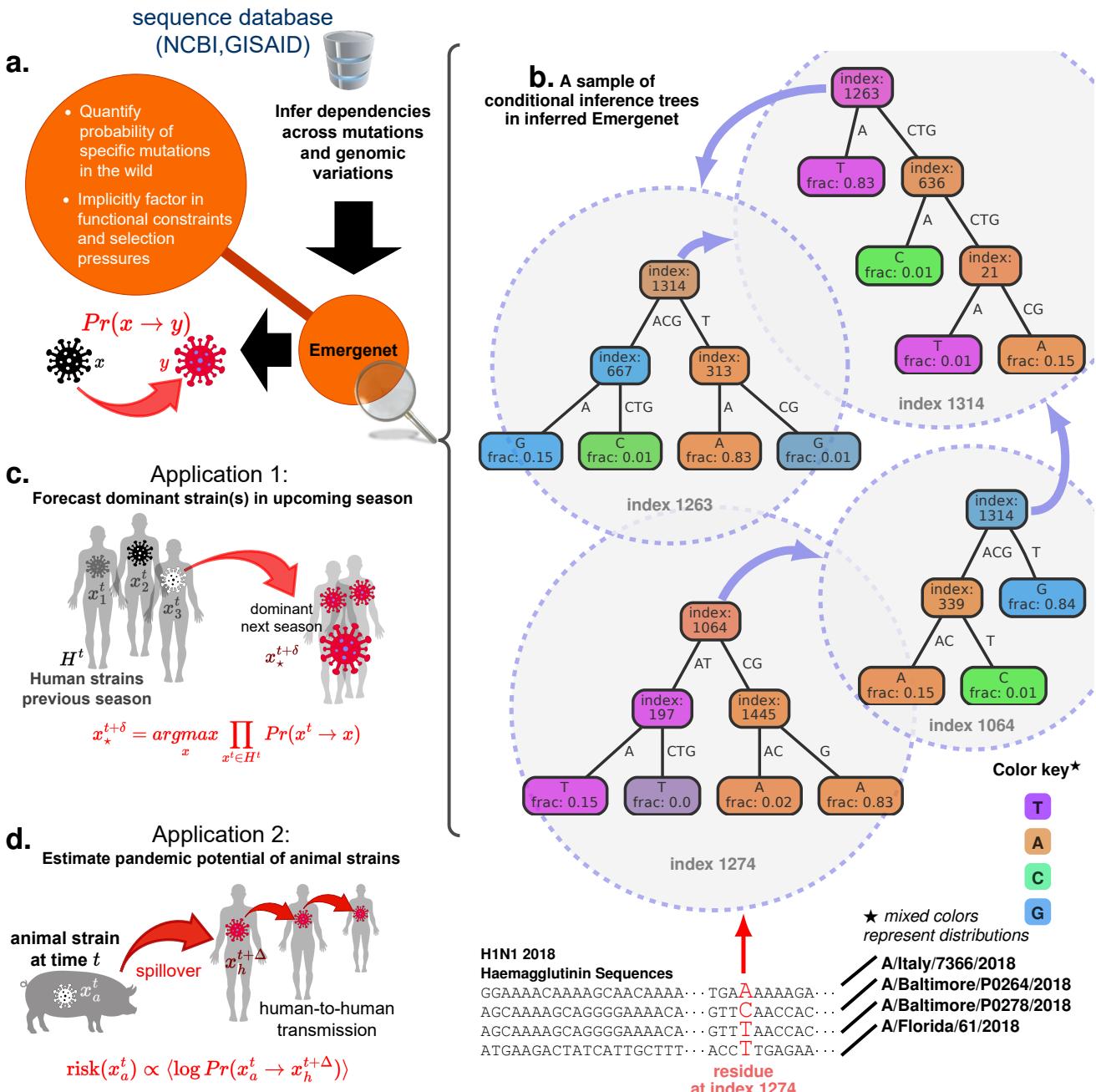
Influenza viruses produce small sequence alterations<sup>1</sup> every season that sufficiently perturb surface protein structures to evade host immunity. Failing to predict the dominant strain in an upcoming season dramatically reduces the effectiveness of the flu shot<sup>2</sup>, yet such prediction remains imperfect. Furthermore, novel strains emerging into humans from animal reservoirs, pose the even graver threat of a global pandemic, and our current ability to scalably evaluate the pandemic potential of individual strains is limited. In this study, we introduce a machine learning algorithm to automatically parse out emergent evolutionary constraints using only genomic sequence data. Analyzing sequences for Hemagglutinin (HA) and Neuraminidase (NA) of nearly 100,000 unique Influenza A strains from public databases, our inferred models (Emergenets) estimate the likelihood of specific future mutations, ultimately yielding the numerical likelihood of specific strains emerging via natural evolutionary processes. We show that our forecasts outperform World Health Organization (WHO) recommended flu vaccine compositions almost consistently over the past two decades, for both H1N1 and H3N2 subtypes, individually in the northern and the southern hemispheres. Additionally, we replicate the Center for Disease Control's (CDC) Influence Risk Assessment (IRAT) score for evaluating the pandemic potential of Influenza A viruses isolated from animal hosts, particularly ones that do not yet circulate in humans. Our substantial speedup (weeks vs seconds) in identifying risky strains is potentially key to fully exploiting the current biosurveillance capacity via scalably analyzing tens of thousands of strains collected every year, thus enabling meaningful preemptive pandemic mitigation strategies.

## INTRODUCTION

Influenza viruses constantly evolve<sup>1</sup>, producing small sequence alterations over a time scale of months that perturb surface protein structures sufficiently to evade the prevailing host immunity, and cause the recurring seasonal flu epidemic. These periodic infection peaks claim a quarter to half a million lives<sup>3</sup> globally, and currently our response comprises yearly innoculation of the population with a reformulated vaccine. Designing the optimal flu shot requires predicting the dominant strain(s) in the upcoming season, and deviations between the predicted and the circulating strain(s) reduce vaccine effectiveness<sup>2</sup> dramatically. Unfortunately, despite recent advances in forecasting tools<sup>3,4</sup>, prediction of the dominant strain(s) remains imperfect.

While the seasonal flu epidemic is a serious health concern, novel Influenza A strains spilling over into humans from animal reservoirs pose the even graver threat of a global pandemic. With the memory of sudden emergence of COVID-19 fresh in our minds, a looming question is whether we can prepare for, preempt and mitigate such events in the future. Influenza A, partly on account of its segmented genome and its wide prevalence in common animal hosts, has historically demonstrated its ability to easily incorporate genes from multiple strains and emerge as novel human pathogens, thus harboring a high potential of triggering the next pandemic.

A possible approach to mitigating such risk is to identify specific strains in animal hosts that do not yet circulate in humans, but have the potential to spill-over and quickly achieve human-to-human (HH) transmission capability. Despite global surveillance efforts to collecting wild specimens from diverse hosts and geo-locations, our current ability to objectively, reliably and scalably evaluate the risk posed to humans by individual strains is limited.



**Fig. 1. Emergenet inference and applications.** **a.**, Variations of genomes for identical subgroups of Influenza A are analyzed to infer a recursive forest of decision trees (conditional inference trees<sup>5</sup>), the Emergenet, which maximally captures the emergent dependencies between an a priori unspecified number of mutations, deletions and insertions. With these inferred dependencies we can estimate the numerical odds of specific mutations, and by extension, the numerical value of the probability of one strain giving rise to another in the wild, under complex selection pressures from the background. **b.**, Snapshot of decision trees from the Emergenet constructed for H1N1 haemagglutinin 2018 sequences. Note that the decision tree predicting the bases at index 1274 uses the bases at 1064, 1445, 197 as features. These features are automatically selected, as being maximally predictive of the bases be at 1274. Then, we compute predictors for each of these feature indices, e.g. trees for index 1064, which involves index 1314 and 339 as features. Continuing, we find that the trees for index 1314 involves indices 1263, 636 and 21, and that for 1263 involves 1314, 667 and 313. The predictor for 1263 depends on 1314, and that for 1314 depends on 1263, revealing the recursive structure of Emergenet. **c.**, First application: With Emergenet induced ability to quantify mutation probabilities, we forecast dominant strain(s) for the next flu season, using only sequences collected in the previous season (and the inferred Emergenet, using data from the past year). **d.**, Second application: estimation of the risk of a global pandemic posed by individual animal strains that are still not known to circulate in humans.

CDC's current, somewhat subjective, solution to this problem is the Influenza Risk Assessment Tool (IRAT), which uses a combination of ten weighted risk elements, aiming to factor in the 1) properties of the virus, 2) attributes of the population, and 3) ecology and epidemiological characteristics of the virus<sup>6</sup> that are largely expert-selected. The IRAT score assigns a grade between 1-10 for emergence risk and public health impact to Influenza A viruses not currently circulating among humans. However, evaluating the IRAT risk element involve multiple experimental assays for each strain, possibly taking weeks to return the final score for a single strain. Thus, we have a scalability problem:

#1. compress this desc to state the key results. Here we are stating what we are doing

while the current global biosurveillance efforts are collecting tens of thousands of sequences every year, most of these sequences will never be analyzed in time. IRAT assessment protocols are not fast enough to leverage the full capacity of current surveillance output, and thus have low odds of preempting a pandemic.

In this study, we introduce a new machine learning algorithm to automatically parse out emergent evolutionary constraints operating on Influenza A viruses in the wild, via analyzing observed variations (point substitutions and indels) in the genomic sequences of key proteins implicated in cellular entry and exit<sup>7</sup> (HA and NA respectively). Uncovering and representing these constraints within a predictive framework, which we call the Emergenet, allows us to estimate the numerical odds of a specific mutation to arise from a given strain spontaneously in the wild within a given time-period. Explicit quantification of these likelihoods then leads to the estimation of the probability of a specific strain of interest giving rise to another, where the odds of such “jumps” are shaped by the inferred emergent constraints.

It is necessary that a viral strain has a sufficiently high replicative fitness for it to be present in sufficiently high numbers in circulation to be observed in the wild. Thus all sequences deposited in the public databases, over which our model trains, may be assumed to have high fitness. It follows that the dependencies between local variations that Emergenet uncovers arises from a functional necessity of maintaining high replicative fitness. However, fitness constraints are not invariant over time especially as the background distribution of strains, and the host immunity changes. With sufficient number of unique samples to train on for each flu season, the Emergenet (recomputed for each time-period) is expected to track these constraints that shape the viral evolutionary trajectories, automatically reflecting the effect of functional constraints, the impact of constantly evolving host immunity, and the current strain distribution in circulation.

The ability of the Emergenet framework to determine the numerical odds of specific sequence variations allows us to frame the problem of forecasting future dominant strain(s), and that of estimating the pandemic potential of an observed animal strain as precise mathematical questions, with demonstrated approximate solutions (Fig. 1). [what are the questions] 1. a dominant strain(s) may be estimated by looking for one that maximizes the probability that all currently circulating strains simultaneously and spontaneously give rise to this target strain. 2. the pandemic risk score of a novel strain is high if it has a high likelihood of spilling over to humans and achieve HH transmission capability.

Structurally, the Emergenet comprises a recursive forest of cross-dependent predictors: each component predictor aims to model the nucleotide “outcome” at a specific positional index, using the sequence entries at other possibly remote parts of the coding sequence as features (Fig. 1b); thus yielding a recursive collection of decision trees. These decision trees are inferred purely from the coding sequences of the proteins of interest with no additional phenotypic annotation other than identifying the host animal. However, probing the deep emergent interdependencies uncovers a rich structure of constraints that informs how strains can evolve while not losing replicative fitness. Our modeling approach is a fundamental departure from the state of the art; we do not assume models of mutation, and we make no *a priori* assumptions that are specific to Influenza A viral sequences. [describe more here]

As our first result, we forecast dominant strain(s) for the seasonal flu, outperforming WHO/CDC recommended flu vaccine compositions almost consistently over the past two decades, for both H1N1 and H3N2 subtypes, individually in the northern and the southern hemispheres. This validates our framework, and outlines an approach to improve the effectiveness of the flu shot, outperforming recent proposals using computational and/or experimental frameworks<sup>3,4</sup>. We show that the Emergenet is able to leverage purely sequence information of specific strains, automatically in conjunction with the background distribution of high fitness strains, to get a substantially better predictive performance to what has been realized by the state of the art, with and without the use of more detailed phenotypic information<sup>3,4</sup>.

#2. Maybe articulate that we can come close to analytical solution to these problems, with equations stating what the optimal solution is.

Our primary claim, however, is the ability to estimate the pandemic potential of novel strains, via a new risk score which we call the Gibb's risk. Our proposed risk score closely tracks the IRAT estimate for Influenza A viruses, specifically ones that do not yet circulate in humans, in seconds as opposed to weeks taken by the detailed experimental assays. This dramatic reduction in time and cost potentially opens the door to fully exploiting the current biosurveillance capacity, scalably analyzing risk from the tens of thousands of strains collected every year, and hence moving towards meaningful preemptive pandemic mitigation strategies.

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Next we would have brief methods, followed by detailed results. Then Discussion, and finally Methods.

Result requirements: 0) Check if changes happen on incorporating the additional term from eqn 9

- 1) percent improvement data table augment with comparison against literature data (in number of average mutational deviation)
- 2) table for fig 2
- 3) table/figure for risk scores of all h3n2/h1n1 strains in swine collected in 2021/2022
- 4) How many sequences were used from which databases. Where is this info described (need this to appear in teh abstract as well)
- 5) all qnets should be submitted to zenodo

#3. review more carefully phenotypic info used in teh literature and why it is claimed to be necessary in those papers. Why dont we need it

## BRIEF METHODS

A key barrier to making progress on both the problems cited above, namely predicting the dominant strain(s) in seasonal flu, as well as estimating the numerical odds of an animal strain to spillover and attain HH capability, is our limited understanding of the emergent dependencies across individual mutations that constrain evolutionary trajectories. Thus, to the best of our knowledge, the state of the art has no tools to estimate the numerical likelihood of specific mutations in the future, and in general the likelihood of a wild strain spontaneously giving rise to another by random chance. Currently, this likelihood is often qualitatively equated to sequence similarity, which is measured by the number of mutations it takes to change one strain to another. However, the odds of one sequence mutating to another is not just a function of how many mutations separate them, but also of how specific mutations incrementally affect fitness. Ignoring the constraints arising from the need to conserve function makes any assessment of the mutation likelihood open to subjective bias. Here, we show that a precise calculation is possible when sequence similarity is evaluated via a new biologically-aware metric, which we call the *q-distance*.

Some recent efforts have recognized this gap, and have attempted to predict future dominant strain by incorporating other phenotypic details.

As an applications of the q-distance, we show that we can improve seasonal forecasts for the future dominant circulating strain by learning from the mutational patterns of key surface proteins: Hemagglutinin (HA) and Neuraminidase (NA) for Influenza A. We outperform the WHO's recommendations for the flu-shot composition consistently over past two decades, measured as the number of mutations that separate the predicted from the dominant circulating strain in each season. Our recommendations repeatedly end up closer to the dominant circulating strain, illustrating the potential of our approach to correctly predict evolutionary trajectories.

We also show that this new metric allows us to assess the risk posed by novel strains effectively and quickly. We compare q-distance results to the CDC's Influenza Risk Assessment Tool (IRAT)<sup>6</sup>, which gives a grade between 1-10 for emergence risk and public health impact to Influenza A viruses not currently circulating among humans. Our results show strong negative correlations between IRAT emergence risk grades and q-distances to the nearest human strains to the strains in question. However, while IRAT may take weeks to analyze a single strain – hence the small number of analyzed strains – q-analysis can be done within milliseconds for each new strain. Moreover, q-analysis only requires sequence data, while IRAT requires information for 10 risk elements, grouped into three categories: 1) properties of the virus, 2) attributes of the population, and 3) ecology and epidemiology of the virus<sup>6</sup>. Thus, our method could potentially be a low-cost, efficient substitute to IRAT, which could be used at scale to rank the risk of emergence of non-circulating strains.

Discussion? Thus, the tool proposed in this study can profoundly impact bio-surveillance strategies. The ability to rank newly collected strains by risk at scale, allows actionable estimates of pandemic risks via quantifying the odds of a particular strain spilling into the human population. Additionally, for strains already circulating in humans, our tools can estimate the odds of specific new variants emerging, and their ability to escape current vaccines.

## METHODS

Aiming to validate our metric in the context of viral evolution, we begin by collecting over 98,000 Influenza A HA/NA nucleotide sequences from two public databases (NCBI and GISAID; see SI-Table 3), uncovering a network of dependencies between individual mutations revealed through subtle variations of the aligned sequences. These dependencies define our organism-specific model referred to as the *quasi-species network* or the Qnet (see Fig. 1 and ??). The q-distance, informed by the dependencies modeled by the inferred Qnets, adapts to the specific organism, allele frequencies, and variations in the background population.

Using aligned genomic sequences sampled from similar populations, e.g. HA from Human Influenza A in year 2008, we construct the Qnet via customized machine learning algorithms to learn models for predicting the mutational variations at each sequence index using other indices as features. For example, in Fig. ??a, the predictor for index 1274 uses

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variation at index 1064 as a feature, and the predictor for index 1064 uses index 1314 as a feature, and so on – ultimately uncovering a recursive dependency structure. The Qnet predicts the nucleotide distribution over the base alphabet at any specific index, conditioned on the nucleotides making up the rest of the sequence of the gene or genome fragment under consideration. Aside from this example, amino acids sequences can also be used to train the Qnet. Finally, we define the q-distance (see Eq. (3) in Materials and Methods) as the square-root of the Jensen-Shannon (JS) divergence<sup>8</sup> of these conditional distributions from one sequence to another, averaged over the entire sequence. Invoking Sanov's theorem on large deviations<sup>8</sup>, we show that the likelihood of spontaneous change is bounded above and below by a simple exponential function of the q-distance.

The mathematical intuition behind relating the new distance to change-probability is the same as in the prediction of a biased outcome when we sequentially toss a fair coin. With an overwhelming probability, such an experiment with a fair coin should result in roughly equal number of heads and tails. However, “large deviations” can happen, and the probability of such rare events is quantifiable<sup>9</sup> with existing theory. We show here that the likelihood of a spontaneous transition of a genomic sequence to a different variant by random chance may also be similarly bounded, given we have the Qnet as an estimated model of the evolutionary constraints.

Importantly, the q-distance between two sequences may change even if only the background population changes (see SI-Table 1, where the distance between two fixed sequences vary when we vary their collection years). Sequences may have a large q-distance and a small edit distance, and vice versa (although on average the two distances tend to be positively correlated, see SI-Table 2). Hence for tracking drift in Influenza A, we construct a seasonal Qnet for each sub-type and protein that we consider.

Our first application aims to predict dominant strains for the seasonal flu epidemic. Periodic adjustment of the Influenza vaccine components is necessary to account for antigenic drift<sup>1,10</sup>. The flu shot in each hemisphere is annually prepared at least six months in advance, and is based on a cocktail of historical strains determined by the WHO via global surveillance<sup>11</sup>, hoping to match the circulating strain(s) in the upcoming flu season. A variety of hard-to-model effects hinder this prediction, which, despite observed cross-reactive effects<sup>2</sup>, have limited vaccine effectiveness in recent years<sup>12</sup>. For predicting future strains, we hypothesized that since the probability of a drift exponentially decreases with an increasing q-distance, the centroid of the strain distribution in our metric will change slowly. If true, the strain selected closest to the “q”-centroid will be a good approximation of next season’s dominant strain. We then computed the dominant strain in each season as the centroid of the strain distribution observed in a given season in the classical sense (no. of mutations), since the edit distance metric is widely used and offers a point of comparison between WHO predictions and Qnet predictions. Note that the recommendations for the northern hemisphere are given in February, while that for the southern hemisphere are given at the end of December the previous year, keeping in mind that the flu season in the south begins a few months early, as described in Fig. 3. Finally, we computed the edit distance (no. of mutations) between the dominant strain and the WHO and Qnet predictions.

Our second application aims to compare emergence risk grades given by the CDC through its Influenza Risk Assessment Tool (IRAT) with results using our q-distance metric. While IRAT uses a combination of 10 weighted risk elements evaluated slowly over the course of several months per strain, we attempt to quantify emergence risk quickly with the q-distance metric. We looked at the same strains that were analyzed by IRAT. For each strain previously analyzed by IRAT, we construct Qnet models for HA and NA segments using all human strains of the same variety circulating in the year prior to risk assessment. For example, the “A/swine/Shandong/1207/2016” strain was assessed by IRAT in July 2020, so we will use human H1N1 strains circulating between July 1, 2019 through June 30, 2020. For sub-types with few human strains (H1N2, H5N1, H5N6, H7N7, H9N2), we only use the upper bound of the date. We then compute the average q-distance between the strain in question and the circulating human strains for both HA and NA segments. Seven of the 23 strains are not included in our comparison due to having zero or too few human strains in the sample space to construct a Qnet; see Supplementary Text, SI-Table 16. We hypothesize that a lower average q-distance between the strain in question and circulating human strains should correspond to a higher emergence risk. Hence, we expect to see a high negative correlation between q-distance and IRAT grade, which assigns 1 to be the lowest risk and 10 to be the highest risk.

## RESULTS

We tested the hypothesis of our first application, computing the strain closest to the “q”-centroid for each flu season and selecting that strain as the prediction for the next season’s dominant strain. We performed this analysis on past two decades of sequence data for Influenza A (H1N1 and H3N2) with promising results: the q-distance based prediction demonstrably outperforms WHO recommendations by reducing the distance between the predicted and the dominant strain (Fig. 3). Recall that we identify the dominant strain to be the one that occurs most frequently, computed as the centroid of the strain distribution observed in a given season in the classical sense (no. of mutations).

The Qnet single-cluster predictions consistently outperform the WHO recommendations. For H1N1 HA, the Qnet induced recommendation outperforms the WHO suggestion by > 33% on average over the last two decades, and > 71% on average in the last decade. The gains for H1N1 NA over the same time periods are > 15% and > 52%, respectively. For H3N2 HA, the Qnet induced recommendation outperforms the WHO suggestion by > 37% on average

TABLE 1  
Out-performance of Qnet recommendations over WHO for Influenza A vaccine composition

Subtype	Gene	Hemisphere	Two decades (% Improvement)	One decade (% Improvement)
H1N1	HA	North	31.78	75.00
H1N1	HA	South	35.02	67.44
H1N1	HA	Average	33.40	71.22
H3N2	HA	North	38.76	42.50
H3N2	HA	South	36.72	38.67
H3N2	HA	Average	37.74	40.58
H1N1	NA	North	19.64	56.00
H1N1	NA	South	11.29	48.28
H1N1	NA	Average	15.46	52.14
H3N2	NA	North	13.92	8.57
H3N2	NA	South	14.77	22.73
H3N2	NA	Average	14.34	15.65

over the last two decades, and > 40% in the last decade. The gains for H3N2 NA over the same time periods are > 14% and > 15%, respectively. Finding multi-cluster predictions has the potential to yield even more improved results, as seen in Fig. 3 and SI-Table 12 through SI-Table 15.

The full table of single-cluster results with improvement broken down by hemisphere is given in Table 1. Fig. 3 illustrates the relative gains computed for both subtypes and the two hemispheres (since the flu season occupy distinct time periods and may have different dominant strains in the northern and southern hemispheres<sup>10</sup>). Additional improvement is possible if we recommend multiple strains every season for the vaccine cocktail (Fig. 3e,f,k,l). The details of the specific strain recommendations made by the Qnet approach for two subtypes (H1N1, H3N2), for two genes (HA, NA) and for the northern and the southern hemispheres over the previous two decades are enumerated in the Supplementary Text in Tables SI-Table 4 through SI-Table 15.

We hypothesized in our second application that there will be a high negative correlation between q-distance and IRAT emergence grade. Plotting our results in Fig. 2, we find a correlation of  $-0.7032$  ( $p < 0.005$ ), which is statistically and substantively significant. We can conclude, therefore, that a lower average q-distance to currently circulating human strains corresponds to a higher risk of emergence with respect to the CDC's grades. Due to the small number of Influenza A strains that have been analyzed by IRAT, we should be wary of the realistic statistical significance of our results. Achieving a moderately high correlation coefficient and p-value is nevertheless a positive result, and further uncovers the potential of our model to quantify risk of emergence.

For further analysis, we also performed q-analysis on IRAT H1- and H3- sub-types by taking average q-distance between the target strain and all human-circulating strains available, with no upper or lower collection date bound. We expected the correlation to be worse than with bounded strains, since a strain being "close" to humans at some point in the past does not necessarily mean being close now. Indeed, our results showed almost no correlation to the IRAT emergence risk scores. Bounded results for H1- and H3- sub-types yielded a correlation of  $-0.6916$ , while unbounded results yielded a correlation of  $0.0545$ ; see SI-Fig. 3.

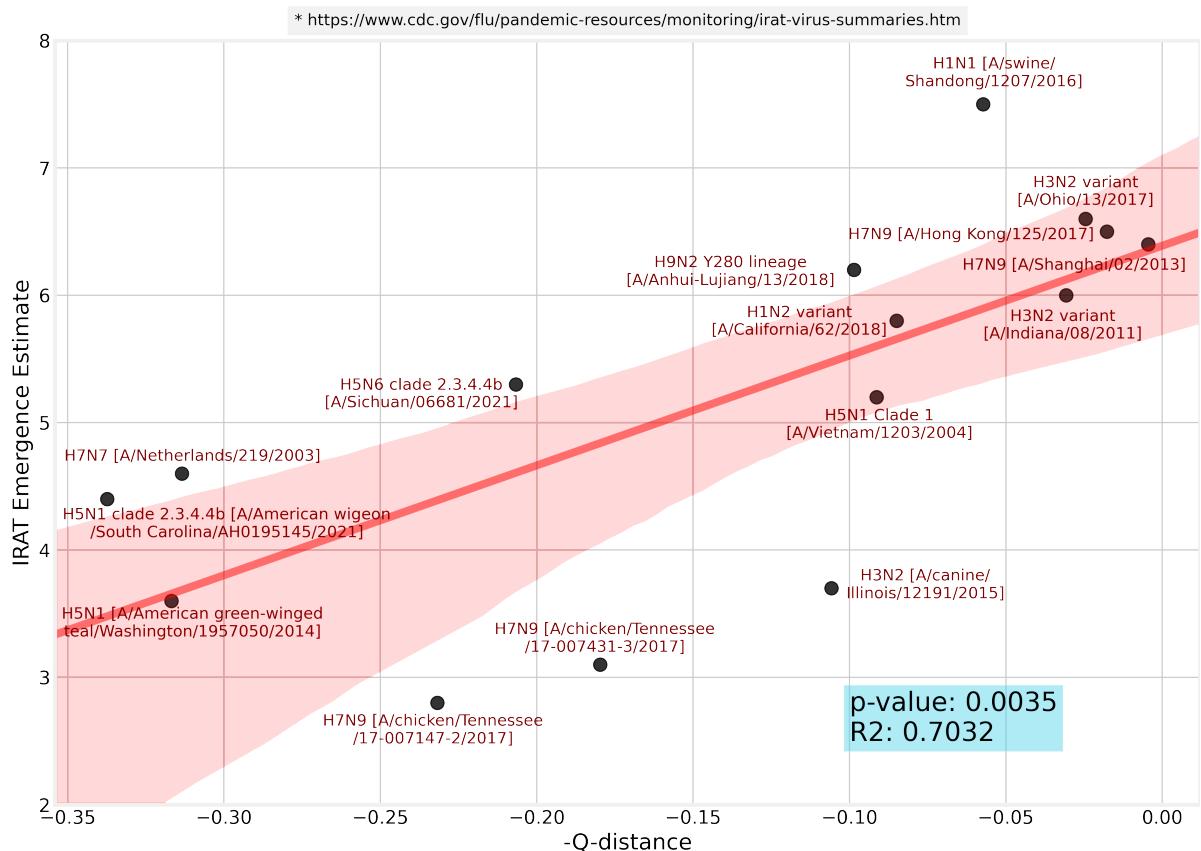
Given the efficiency of the q-distance computations, we can track how risk of emergence changes over time by continually updating the current human-circulating strains each year. For exact average q-distance and Qnet sample size statistics, please see SI-Table 16 in the Supplementary Text.

## EMERGENET ADVANTAGES AND RELATED LITERATURE

Numerous tools exist for ad hoc quantification of genomic similarity<sup>4,13–17</sup>, which are not inherently biologically meaningful – a smaller edit distance between two strains does not necessarily imply that a feasible trajectory exists from one to the other in the wild. These measures tend to be variations of distances between symbolic sequences, and are not aware of selection pressures and evolutionary dynamics. Despite the diverse techniques and concepts explored in these domains, the key missing piece is effectively learning which changes are likely in the wild, conditioned on possibly the entire sequence of the current strain. Our algorithm is the first of its kind to learn an appropriate metric of comparison from data, without assuming any model of DNA or amino acid substitution, or a genealogical tree a priori, and is designed to be aware of the impact of the host environment and background epidemiology.

This is a major improvement over the existing state of art in phylogeny construction from sequences, which generally assume a model for character substitution (either for nucleotides or amino acid residues) ignoring the effect of selection and the existence of long-range complex dependencies in viable mutations along the genomic sequence. Notably, even relatively complex substitution models (*e.g.* ones that allow site specific mutation rates) do not capture the effect of individual changes that may dramatically alter fitness in the environment. Our proposed approach, on the other

### CDC-published IRAT\* vs Q-distance



**Fig. 2. IRAT emergence risk vs. q-distance.** There is an approximate linear relationship between average q-distance from human circulating strains (averaged across both HA and NA) and IRAT emergence risk grade. Note that IRAT has released results for 23 strains to date, but only 15 are plotted on the graph. This is because the strains not pictured have less than 30 human strains of the same sub-type, so a sufficiently representative Qnet could not be trained.

hand, learns from and leverages these patterns, using sophisticated pattern discovery via novel machine learning algorithms. While the effect of the environment and selection cannot be inferred from a single sequence, an entire database of observed strains, processed through sophisticated learning, can parse out predictive models of these complex interactions. Our only intuitively well-justified assumption on the evolutionary dynamics is that more fit strains end up with more progenies (follows from definition of Malthusian fitness), and are thus more likely to be sampled in surveillance efforts (again, intuitively obvious). Thus, in a strict mathematical sense, the distance we propose is not a distance between two strains  $x, y$ , but a distance between strain  $x^A$  ( $x$  in a background environment  $A$ ) and a strain  $y^B$  ( $y$  in a background environment  $B$ ). Indeed we can show that the distance between the same pair of strains of Influenza A HA is different based on if they are collected in 2008 vs in 2009, reflecting that the background environment and circulating diversity changed over the two years. Thus, our distance metric is fundamentally different from measures that exist in the literature. In particular, our mathematical framework leads to the key result that the q-distance is a scaled representation of the log-likelihood of spontaneous jump between strains. This interpretation is missing in existing tools, and makes way for leveraging the q-distance to model emergence of new strains. Thus, we can predict entirely new sequences – which differ by a non-trivial number of edits from any observed strain – that still lead to functional proteins, as demonstrated in our preliminary studies.

Very recently, two articles have explored the possibility of predicting pathogenicity from genomic sequences (Mollentze<sup>18</sup>) and forecasting which amongst observed mutations will dominate the circulating population (Maher et al<sup>19</sup>). These studies provide strong pieces of evidence that challenge the idea that forecasting future variants of virus strains is impossible, while aligns with our goals. While their questions overlap with our framework, our approach is distinct and more ambitious. For example, Mollentze uses classical sequence similarity; extended to include similarity to human housekeeping genes hoping to identify viruses evading the human immune system more easily. The demonstrated performance is poor (incorrectly tagging all SARS-related coronaviruses as potentially pathogenic), implying unactionable specificity. On the other hand, Maher outright assumes mutations to be independent. Features are found manually, are specific to SARS-CoV-2, and the authors take a meta-analysis-esque route, compiling together a “kitchen-sink” of features via standard machine learning. Importantly, these approaches only aim to predict point mutations, with the gargantuan complexity of tracking a more complete strain through a high-dimensional sequence space well beyond their conceptual limits. Thus, even the question if whether a yet-to-be-seen strain is indeed a valid biological encoding of a virus (which is simpler to determining risk posed by such future variants) cannot be answered by our peers,

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limiting such approaches to analyzing mutations already seen, or strains already collected. Additionally, generalizability and actionability is suspect, given that Maher's features are SARS-CoV-2 specific, and Mollentze's similarity to house-keeping genes might not be universal. Finally, both these approaches apply to a mutation or a combination of mutations that already exist, and cannot predict new mutations, or new strains.

## DISCUSSION

In the aftermath of the COVID-19 pandemic that caused one of the most devastating disasters of the past century, a looming question is whether we can prepare for, preempt and mitigate such events in the future. Evolving viruses, whether currently circulating in the human population, or in animal reservoirs that might spillover and attain human-to-human transmission capability, pose an ever-present epidemic risk. Current surveillance paradigms, while crucial for mapping disease ecosystems, are limited in their ability to address this challenge. Habitat encroachment, climate change, and other ecological factors<sup>20–22</sup> unquestionably drive up the odds of zoonotic spill-overs. Nevertheless, current efforts at tracking these effects have not improved our ability to quantify future risk of emergence<sup>23</sup>. Tracking viral diversity in animal hosts, while important, often does not transparently map to emergence risk. This is particularly true for Influenza A, which partly on account of its segmented genome, can easily incorporate genes from multiple strains and emerge as novel human pathogens, and thus harbor a high pandemic potential. While large antigenic shifts in Influenza A are relatively rare, even the smaller seasonal sequence alterations in cause sufficient variation in the surface proteins to evade existing immunity, and require yearly reformulation of the flu vaccine.

However, for the vaccine to be effective, we need to predict the dominant circulating strain of the upcoming season with sufficient accuracy. Currently, the composition of the flu shot is decided at least six months in advance of the seasonal infection peak, and targets three to four historical strains as recommended by the CDC/WHO, who identify these specific strains by sampling the current circulation<sup>11</sup>, hoping to match the dominant strain(s) in the upcoming season. A variety of hard-to-model effects hinder this prediction, which, despite observed cross-reactive effects<sup>2</sup>, have had limited vaccine effectiveness in recent years<sup>12</sup>. Rank-ordering strains which do not yet circulate in humans according to either their spillover risk or their pandemic potential, has proven to be even more difficult [REF]. CDC's current, somewhat subjective, solution to this problem is the Influenza Risk Assessment Tool (IRAT), which uses a combination of ten weighted risk elements, including 1) properties of the virus, 2) attributes of the population, and 3) ecology and epidemiological characteristics of the virus<sup>6</sup> that are expert-selected. Evaluating these factors involve several experimental assay for each strain, taking possibly weeks to return the final IRAT score for a single strain. Thus, we have a scalability problem: with the current global biosurveillance efforts collecting tens of thousands of sequences every year, IRAT assessment is simply not fast enough to preempt a pandemic.

## DISCUSSION & SEQUENCE COMPARISONS

For further discussion, we looked at our Qnet predictions more closely. Comparing the Qnet inferred strain (QNT) against the one recommended by the WHO, we find: 1) the residues that only the QNT matches correctly with DOM (while the WHO fails) are largely localized within the receptor binding domain (RBD), with > 57% occurring within the RBD on average (see Fig. 4a for a specific example), and 2) when the WHO strain deviates from the QNT/DOM matched residue, the “correct” residue is often replaced in the WHO recommendation with one that has very different side chain, hydropathy and/or chemical properties (see Fig. 4b-f), suggesting deviations in recognition characteristics. Combined with the fact that we find circulating strains are almost always within a few edits of the DOM (see SI-Fig. 2), these observations suggest that hosts vaccinated with the QNT recommendation is more likely to have season-specific antibodies that are more likely to recognize a larger cross-section of the circulating strains.

High season-to-season genomic variation in the key Influenza capsidic proteins is driven by two opposing influences: 1) the need to conserve function limiting random mutations, and 2) hyper-variability to escape recognition by neutralizing antibodies. Even a single residue change in the surface proteins might dramatically alter recognition characteristics, brought about by unpredictable<sup>24,25</sup> changes in local or regional properties such as charge, hydropathy, side chain solvent accessibility<sup>26–29</sup>.

Focusing on the average localization of the QNT to WHO deviations in the HA molecular structure, the changes are observed to primarily occur in the HA1 sub-unit (see Fig. 4g-i, HA0 numbering used, other numbering conversions are given in SI-Table 18), with the most frequent deviations occurring around the ≈ 200 loop, the ≈ 220 loop, the ≈ 180 helix, and the ≈ 100 helix, in addition to some residues in the HA2 sub-unit (≈ 49 & ≈ 124). Unsurprisingly, the residues we find to be most impacted in the HA1 sub-unit (the globular top of the fusion protein) have been repeatedly implicated in receptor binding interactions<sup>30–32</sup>. Thus, we are able to fine tune the future recommendation over the state of the art, largely by modifying residue recommendations around the RBD and structures affecting recognition dynamics.

## LIMITATIONS & CONCLUSION

Calculation of q-distance is currently limited to similar and aligned sequences, *e.g.* Influenza strains from different sub-types, hosts or seasons. Furthermore, we need a sufficient diversity of observed strains to successfully construct the Qnet. A multi-variate regression analysis indicates that the most important factor for our approach to succeed is the diversity of the sequence dataset (see Supplementary Text, SI Table 17). Arguably, simply reducing the edit distance from the dominant strain is not guaranteed to translate to a better immunological protection. Nevertheless, consistent improvement in this metric achieved purely via computational means suggests the possibility of improvement over current practice.

In conclusion, we introduce a data-driven distance metric to track subtle deviations in sequences. We show that we can use the q-distance metric to make recommendations for the flu-shot composition, outperforming the WHO's recommendations in relation to the dominant strain. We also show that we can roughly replicate the CDC's IRAT grades for emergence risk of strains not currently circulating among humans in an efficient manner that can be scaled to rank many more strains than is currently done. The ability to predict future flu strains via subtle variations in a limited set of immunologically important residues suggest that the tools developed here could lead to more effective escape-resistant vaccines, which could be essential in preempting and mitigating the next pandemic.

Next, we briefly describe the details of the computational framework.

## EMERGENET FRAMEWORK

We do not assume that the mutational variations at the individual indices of a genomic sequence are independent (See Fig 1a). Irrespective of whether mutations are truly random<sup>33</sup>, since only certain combinations of individual mutations are viable, individual mutations across a genomic sequence replicating in the wild appear constrained, which is what is explicitly modeled in our approach. The mathematical form of our metric is not arbitrary; JS divergence is a symmetricised version of the more common KL divergence<sup>8</sup> between distributions, and among different possibilities, the E-distance is the simplest metric such that the likelihood of a spontaneous jump (See Eq. (4) in Methods) is provably bounded above and below by simple exponential functions of the E-distance.

Consider a set of random variables  $X = \{X_i\}$ , with  $i \in \{1, \dots, N\}$ , each taking value from the respective sets  $\Sigma_i$ . A sample  $x \in \prod_1^N \Sigma_i$  is an ordered  $N$ -tuple, consisting of a realization of each of the variables  $X_i$  with the  $i^{th}$  entry  $x_i$  being the realization of random variable  $X_i$ . We use the notation  $x_{-i}$  and  $x^{i,\sigma}$  to denote:

$$x_{-i} \triangleq x_1, \dots, x_{i-1}, x_{i+1}, \dots, x_N \quad (1a)$$

$$x^{i,\sigma} \triangleq x_1, \dots, x_{i-1}, \sigma, x_{i+1}, \dots, x_N, \sigma \in \Sigma_i \quad (1b)$$

Also,  $\mathcal{D}(S)$  denotes the set of probability measures on a set  $S$ , *e.g.*,  $\mathcal{D}(\Sigma_i)$  is the set of distributions on  $\Sigma_i$ .

We note that  $X$  defines a random field<sup>34</sup> over the index set  $\{1, \dots, N\}$ . To clarify the biological picture, we refer to the sample  $x$  as an amino acid or nucleotide sequence, identifying the entry at each index with the corresponding protein residue or the nucleotide base pair.

**Definition 1** (Emergenet). *For a random field  $X = \{X_i\}$  indexed by  $i \in \{1, \dots, N\}$ , the Emergenet is defined to be the set of predictors  $\Phi = \{\Phi_i\}$ , i.e., we have:*

$$\Phi_i : \prod_{j \neq i} \Sigma_j \rightarrow \mathcal{D}(\Sigma_i), \quad (2)$$

where for a sequence  $x$ ,  $\Phi_i(x_{-i})$  estimates the distribution of  $X_i$  on the set  $\Sigma_i$ .

We use conditional inference trees as models for predictors<sup>5</sup>, although more general models are possible.

## Biology-Aware Distance Between Sequences

**Definition 2** (E-distance: adaptive biologically meaningful dissimilarity between sequences). *Given two sequences  $x, y \in \prod_1^N \Sigma_i$ , such that  $x, y$  are drawn from the populations  $P, Q$  inducing the Emergenet  $\Phi^P, \Phi^Q$ , respectively, we define a pseudo-metric  $\theta(x, y)$ , as follows:*

$$\theta(x, y) \triangleq \mathbf{E}_i \left( \mathbb{J}^{\frac{1}{2}} \left( \Phi_i^P(x_{-i}), \Phi_i^Q(y_{-i}) \right) \right) \quad (3)$$

where  $\mathbb{J}(\cdot, \cdot)$  is the Jensen-Shannon divergence<sup>35</sup> and  $\mathbf{E}_i$  indicates expectation over the indices.

The square-root in the definition arises naturally from the bounds we are able to prove, and is dictated by the form of Pinsker's inequality<sup>8</sup>, ensuring that the sum of the length of successive path fragments equates the length of the path, making it possible to use standard algorithms for q-phylogeny construction.

## Theoretical Probability Bounds

The Emergenet framework allows us to rigorously compute bounds on the probability of a spontaneous change of one strain to another, brought about by chance mutations. While any sequence of mutations is equally likely, the “fitness” of the resultant strain, or the probability that it will even result in a viable strain, is not. Thus the necessity of preserving function dictates that not all random changes are viable, and the probability of observing some trajectories through the sequence space are far greater than others. The Emergenet framework allows us to explore this constrained dynamics, as revealed by a sufficiently large set of genomic sequences.

We show in Theorem 1 in the supplementary text that at a significance level  $\alpha$ , with a sequence length  $N$ , the probability of spontaneous jump of sequence  $x$  from population  $P$  to sequence  $y$  in population  $Q$ ,  $Pr(x \rightarrow y)$ , is bounded by:

$$\omega_y^Q e^{\frac{\sqrt{8N^2}}{1-\alpha} \theta(x, y)} \geq Pr(x \rightarrow y) \geq \omega_y^Q e^{-\frac{\sqrt{8N^2}}{1-\alpha} \theta(x, y)} \quad (4)$$

where  $\omega_y^Q$  is the membership probability of strain  $y$  in the target population.

The ability to estimate the probability of spontaneous jump between sequences in terms of  $\theta$  has crucial implications. It allows us to 1) construct a new phylogeny that directly relates the probability of jumps rather than the number of mutations between descendants. 2) simulate realistic trajectories in the sequence space from any given initial strain, and 3) estimate drift in the sequence space by analyzing the statistical characteristics of the diffusion occurring in the strain space.

## Application 1: Predicting Seasonal Strains

Analyzing the distribution of sequences observed to circulate in the human population at the present time allows us to forecast dominant strain(s) in the next flu season as follows:

Let  $x_*^{t+\delta}$  be a dominant strain in the upcoming flu season at time  $t + \delta$ , where  $H^t$  is the set of observed strains presently in circulation in the human population (at time  $t$ ). We will assume that the Emergenet remains unchanged upto  $t + \delta$ . From the RHS bound established in Theorem 1 (See Eq. (4) above) in the supplementary text, we have:

$$\ln \frac{Pr(x \rightarrow x^{t+\delta})}{\omega_{x^{t+\delta}}} \geq -\frac{\sqrt{8N^2}}{1-\alpha} \theta(x, x^{t+\delta}) \quad (5)$$

$$\Rightarrow \sum_{x \in H^t} \ln \frac{Pr(x \rightarrow x^{t+\delta})}{\omega_{x^{t+\delta}}} \sum_{x \in H^t} \geq -\frac{\sqrt{8N^2}}{1-\alpha} \theta(x, x^{t+\delta}) \quad (6)$$

$$\Rightarrow \sum_{x \in H^t} \theta(x, x^{t+\delta}) - |H^t| A \ln \omega_{x^{t+\delta}} \geq A \ln \frac{1}{\prod_{x \in H^t} Pr(x \rightarrow x^{t+\delta})} \quad (7)$$

where  $A = \frac{1-\alpha}{\sqrt{8N^2}}$ , where  $N$  is the sequence length considered, and  $\alpha$  is a fixed significance level. Since minimizing the LHS maximizes the lower bound on the probability of the observed strains simultaneously giving rise to  $x^{t+\delta}$ , a dominant strain  $x_*^{t+\delta}$  may be estimated as a solution to the optimization problem:

$$x_*^{t+\delta} = \arg \min_{y \in \cup_{\tau \leq t} H^\tau} \sum_{x \in H^t} \theta(x, y) - |H^t| A \ln \omega_y \quad (8)$$

Further noting that the second term on the right is at least an order of magnitude smaller compared to the first term, a good approximation for the optimization may be stated as:

$$x_*^{t+\delta} = \arg \min_{y \in \cup_{\tau \leq t} H^\tau} \sum_{x \in H^t} \theta(x, y) \quad (9)$$

## Application 2: Measure of Pandemic Potential

We measure the potential of an animal strain  $x_a^t$  to spillover and become HH capable as a human strain  $x_h^{t+\delta}$ , as follows:

$$\rho(x_a^t) \triangleq -\frac{1}{|H^t|} \sum_{x \in H^t} \theta(x_a^t, x) \quad (10)$$

The intuition here is that a lower bound of  $\rho(x_a^t)$  scales as average log-likelihood of the  $x_a^t$  giving rise to a human strains in circulation at time  $t$ . Since the strains in  $H^t$  are already HH capable, a high average likelihood of producing a similar strain has a high potential of being a HH cabale novel variant, which is a necessary condition of a pandemic strain. To establish the lower bound, we note that from Theorem 1 (See Eq. (4) above) in the supplementary text, we have:

$$\sum_{y \in H^t} \ln \left| \frac{Pr(x_a^t \rightarrow y)}{\omega_y} \right| \leq -\frac{\sqrt{8N^2}}{1-\alpha} |H^t| \rho(x_a^t) \quad (11)$$

Denoting,  $A = \frac{1-\alpha}{\sqrt{8N^2}}$ ,  $A \ln(\prod_{y \in H^t} \omega_y) = C$ , and  $\langle \cdot \rangle$  as the geometric mean function, we have:

$$\Rightarrow \rho(x_a^t) \geq A \ln \left( \prod_{y \in H^t} Pr(x_a^t \rightarrow y) \right)^{1/|H^t|} + C \quad (12)$$

$$\Rightarrow \rho(x_a^t) \geq A \ln \left\langle Pr(x_a^t \rightarrow x_h^{t+\delta}) \right\rangle + C \quad (13)$$

Noting that  $A, C$  are not functions of  $x_a^t$ , we conclude that the risk measure  $\rho(\cdot)$  scales with the average loglikelihood of producing strains close to a circulating human strain at the current time.

## DATA SHARING

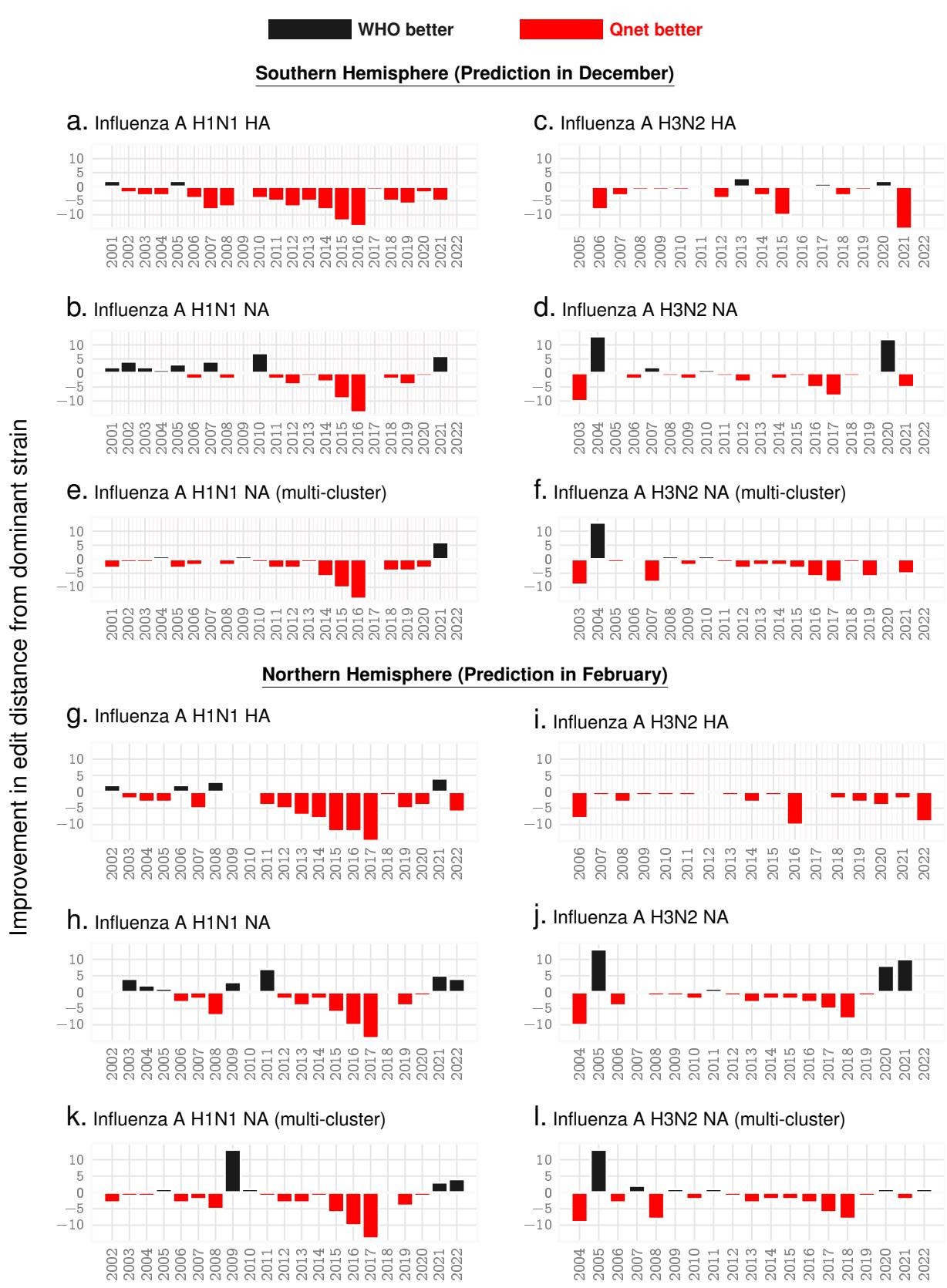
Working software is publicly available at <https://pypi.org/project/emergenet/>. Accession numbers of all sequences used, and acknowledgement documentation for GISAID sequences is available as supplementary information.

### Data Source

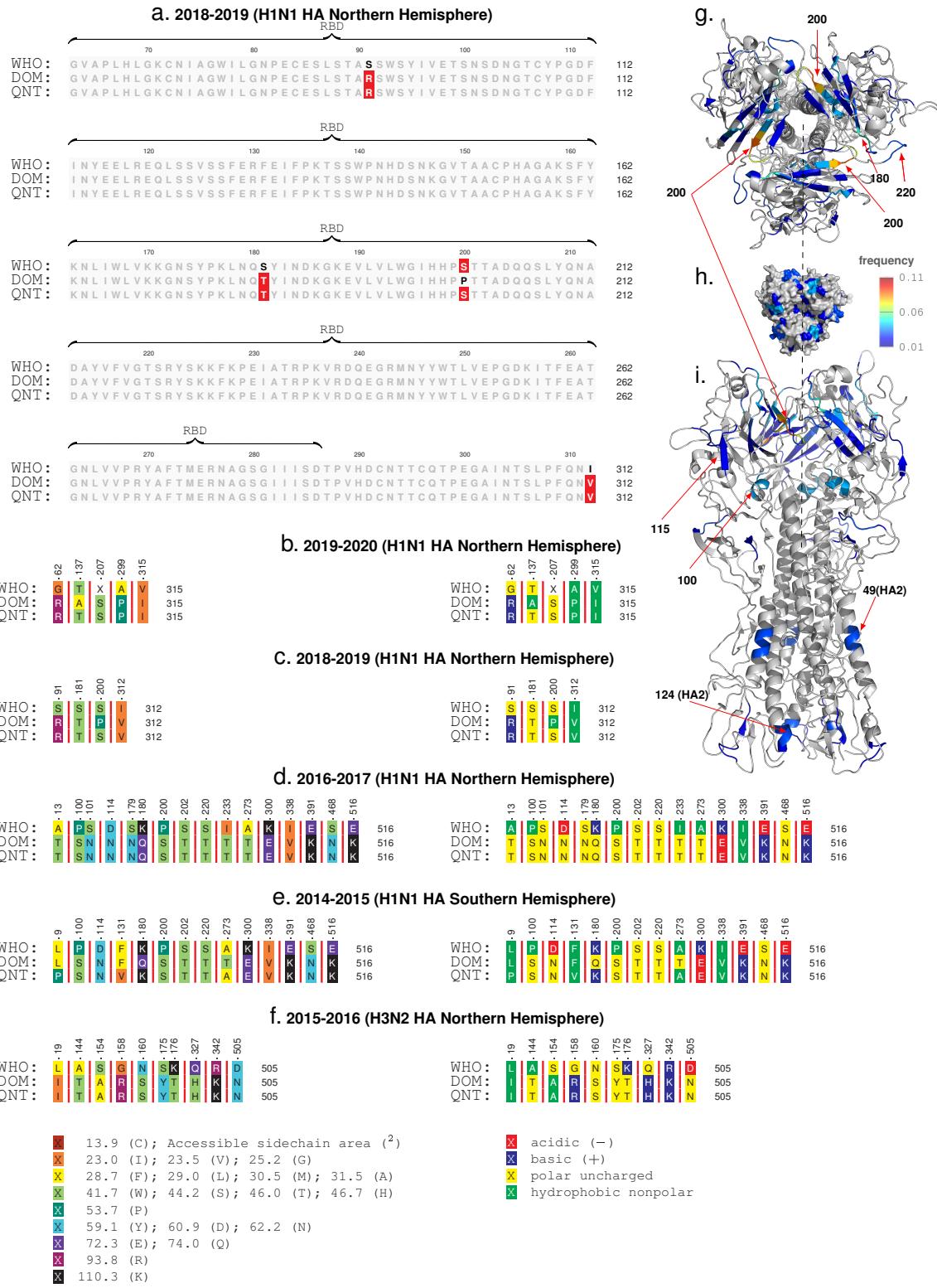
In this study, we use sequences for the Hemagglutinin (HA) and Neuraminidase (NA) for Influenza A (for subtypes H1N1 and H3N2), which are key enablers of cellular entry and exit mechanisms respectively<sup>36</sup>. We use two sequences databases: 1) National Center for Biotechnology Information (NCBI) virus<sup>37</sup> and 2) GISAID<sup>38</sup> databases. The former is a community portal for viral sequence data, aiming to increase the usability of data archived in various NCBI repositories. GISAID has a somewhat more restricted user agreement, and use of GISAID data in an analysis requires acknowledgment of the contributions of both the submitting and the originating laboratories (Corresponding acknowledgment tables are included as supplementary information). We collected a total of 98,299 sequences in our analysis, although not all were used due to some being duplicates (see SI-Table 3).

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**Fig. 3. Seasonal predictions for Influenza A.** Relative out-performance of Qnet predictions against WHO recommendations for H1N1 and H3N2 sub-types for the HA and NA coding sequences over both hemispheres. The negative bars (red) indicate the reduced edit distance between the predicted sequence and the actual dominant strain that emerged that year. Note that the recommendations for the north are given in February, while that for the south are given at the previous December, keeping in mind that the flu season in the south begins a few months early (e.g. for the 2021-2022 flu season, southern data in the table is labelled '2021' and northern is labelled '2022'). **Panels e, f, k, l** show further possible improvement in NA predictions if we return three recommendations instead of one each year.



**Fig. 4. Sequence comparisons.** The observed dominant strain, we note that the correct Qnet deviations tend to be within the RBD, both for H1N1 and H3N2 for HA (panel a shows one example). Additionally, by comparing the type, side chain area, and the accessible side chain area, we note that the changes often have very different properties (panel b-f). Panels g-i show the localization of the deviations in the molecular structure of HA, where we note that the changes are most frequent in the HA1 sub-unit (the globular head), and around residues and structures that have been commonly implicated in receptor binding interactions e.g the ≈ 200 loop, the ≈ 220 loop and the ≈ 180-helix.

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