Algorithmic Bio-surveillance For Precise Spatio-temporal Prediction of Zoonotic Emergence

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Abstract—Viral zoonoses have emerged as the key drivers of recent pandemics. Human infection by zoonotic viruses are either spillover events – isolated infections that fail to cause a widespread contagion – or species jumps, where successful adaptation to the new host leads to a pandemic. Despite expensive bio-surveillance efforts, historically emergence response has been reactive, and post-hoc. Here we use machine inference to demonstrate a high accuracy predictive bio-surveillance capability, designed to proactively localize an impending species jump via automated interrogation of massive sequence databases of viral proteins. Our results suggest that a jump might not purely be the result of an isolated unfortunate cross-infection localized in space and time; there are subtle yet detectable patterns of genotypic changes accumulating in the global viral population leading up to emergence. Using tens of thousands of protein sequences simultaneously, we train models that track maximum achievable accuracy for disambiguating host tropism from the primary structure of surface proteins, and show that the inverse classification accuracy is a quantitative indicator of jump risk. We validate our claim in the context of the 2009 swine flu outbreak, and the 2004 emergence of H5N1 subspecies of Influenza A from avian reservoirs; illustrating that interrogation of the global viral population can unambiguously track a near monotonic risk elevation over several preceding years leading to eventual emergence.

Index Terms—bio-surveillance, Influenza A, antigenic shift, pandemic

Emerging human diseases are often infections caused by pathogens of animal origin[1],[2] (zoonoses). Identification of high-risk pathogens within animal hosts can be used to proactively trigger mitigation strategies, potentially reducing the risk of a successful jump to humans. However, our incomplete understanding of host-pathogen interaction hinders preemptive recognition of subtle signals that elevate the jump risk. A complex interplay of the standing viral population, animal and human hosts, environmental and socio-economic factors, make the task of identifying viruses of high zoonotic or pandemic risk, before emergence, difficult to uncertain at best.[5-19]

Here we present an efficient, data-driven approach to persistent predictive bio-surveillance. At the core of our approach is an inference algorithm to estimate dissimilarity between distinct viral populations, viewed as ensembles of protein sequences. In contrast to distance calculations in phylogenetic analyses, where one computes a distance between two individual sequences,[10]-[14] here we compute the dissimilarity or distance between two sequence ensembles. Unlike static distance formulae, our measure adapts to the evolving populations to back out the most important set of disambiguating residues (features) for the two populations. Computing, in this manner, the instantaneous dissimilarity between the host-specific viral quasi-species leads us to a time-varying measure of jump risk. As an example, we claim that greater the similarity between the population of human influenza viruses and those currently prevalent in swines, higher the possibility of a species jump.

In machine learning parlance, our algorithm trains a classifier: given two sets of amino acid sequences for a specific viral protein corresponding to the two host species, it infers the optimal set of decision rules that disambiguate the populations with maximum achievable accuracy. Then, dissimilarity is simply the inverse accuracy for the learned model. The interpretation here is the tautology that “similar” objects are harder to distinguish, and hence lower classification accuracy indicates a higher degree of similarity. The inferred classifier evolves with time, always distilling the optimal set of disambiguating rules to separate the populations. This adaptive tracking of the evolutionary changes, along with the elimination of the choice of which static distance to use, provides us with a more natural framework to discern subtle changes across viral populations.

Key Insight

With the application of the inverse classification accuracy in estimating jump risk, we are putting forward (and eventually validating) a key hypothesis: emergence risk may be estimated accurately by looking for subtle sequence changes over time in circulating strains. Underlying conventional post-hoc reconstruction of emergence pathways, there is the assumption that species jumps are the result of an unfortunate sequence of antigenic shifts — abrupt genetic rearrangements between distinct strains co-infecting the same host cell, that dramatically alter the antigenic makeup of the resultant virus. Our hypothesis, if true, would imply that such reconstructions do not convey a complete picture of the processes and interactions that foster emergence.

The 2009 pandemic strain (pH1N1) serves as a good example. The emergent strain became known as “swine flu”, on account of pH1N1’s strong similarities with the then circulating swine influenza viruses; phylogenetic analyses showed that the pH1N1 genes clustered with those from swine viruses rather than the seasonal human flu strains. Further analysis suggested that pH1N1 resulted from the re-assortment of 2, or even 3, distinct viruses, namely the Eurasian swine H1N1, and the swine H1N2: the latter itself having emerged from swine H1N1 and the triple assortment swine strain trH3N2, which in turn had contributions from the human H3N2 (related to the Hong Kong flu epidemic of 1968), and even had similarities to avian strains circulating in north America.[17],[19] It is generally recognized that such reconstructions of evolutionary pathways are not unique. Alternate event sequences might have transpired in practice, particularly since swine H1-containing viruses regularly spill-over to humans without causing widespread infections. Additionally, while all pH1N1 genes appear to have originated in swines, they come from geographically widely distributed ancestors.
Fig. 1. **Main Results.** Automated inference of emergent patterns in host-specific HA and NA protein sequences (targeting human, swine and avian hosts) from the Influenza Research Database (IRD), distills an algorithmic risk predictor for zoonotic emergence for influenza. Plates A and B illustrate risk inference for the cases of the 2009 swine flu and the 2004 H5N1 emergence events. In both cases, we see a near monotonic risk elevation leading up to the event, with multiple years of actionable warning. *Importantly, the inference algorithm only uses past information at each predicted time-point.* Except for small variations in accuracy, similar results are obtained for both HA and NA sequences. This is not surprising: while NA is not directly implicated in cellular entry, it is known to assist in transmission via enabling release of progeny viruses.\(^{15}\) Our algorithm is not specific to influenza, and is applicable generally for predicting zoonotic emergence. Plate C compares the predicted H5N1 emergence risk (appropriately scaled) to incidence reported by WHO.\(^{16}\) We shifted the risk plot by 1 year into future to illustrate the close match, i.e., our prediction closely pre-empted the overall incidence dynamics (positive correlation of 0.88 (with death counts) with p-value less than 0.0001). Plate D illustrates the correlation between residue specific standard deviations for host pairs as they evolve over time, where we use the same set of residues (See Table 2) as identified by our algorithm to have predictive value. We note that the swine-human and avian-human correlations are significant, while the avian-human is not; potentially corroborating the idea of domestic pigs as mixing vessels\(^{17},\)\(^{18}\) (See Discussion).

One explanation to this ancestral diversity is the possibility that pH1N1 emerged over a span several years, cryptically circulating in swines before pandemic recognition.\(^{17}\) Irrespective of the specific details, if antigenic shifts are solely responsible for species jumps, then emergence is precipitated entirely by chance events; and hence is categorically impossible to predict — even with vast surveillance efforts. In contrast, our hypothesis suggests that gradual processes, such as antigenic drift brought about by point mutations continuously altering the transcribed proteins over time, play a crucial role; in essence setting up the stage for the re-assortment event that leads to emergence.

Our risk indicator does not require identification of the specific originating animal. Global sampling of the host-specific viral populations suffices to track the progressive similarity of the populations, and a near monotonic risk elevation leading up to the jump. If we need to somehow locate the specific animal(s) in which a new virus emerges in time — every time — then, it is ultimately a losing battle. For example, the 2009 pandemic strain was isolated in a specific pig farm months after the first reported human infections.\(^{19}\) However, if we can reliably estimate jump risk in space, time and originating species by merely sampling animals across the globe, and individual members of the host species are less important, then we shift the odds in our favor.
Quantifying Jump Risk For Influenza A

Influenza is responsible for one of the most devastating epide-
memics in human history, decimating over 2% of the human
population in the H1N1 Spanish flu outbreak of 1918-1920. In
addition to be implicated in tens of thousands of deaths every
year in US alone from the recurring seasonal flu epidemic,
influenza continues to emerge again and again in humans from
strains circulating in animals, leading to severe to moderate
spikes in incidence and mortality rates. Two such recent pan-
demics are the 2004 emergence of the highly pathogenic H5N1
avian strain, and the pH1N1 swine flu outbreak of 2009. Given
the fact that all known influenza subtypes have been isolated in
birds [22]-[24] and that all pandemics with the exception of the
2009 event were caused by strains of avian origin,[19] surveilling
avian strains is of paramount importance. With the emergence of
pH1N1 with its complicated genetic ancestry causing between
151,700 and 575,400 deaths,[25] and that all pandemics with the exception of the
2009 event were caused by strains of avian origin,[19] surveilling
avian strains is of paramount importance. With the emergence of
pH1N1 with its complicated genetic ancestry causing between
151,700 and 575,400 deaths,[25] it is also imperative that we
monitor swines for future emergence. These recent events,
along with the availability of large databases of influenza
proteins (Influenza Research Database or IRD [26]), prompted
us to select avian and swine Influenza A viruses as validation
candidates for our general bio-surveillance algorithm.

Influenza A is a negative stranded RNA virus with an en-
capsulated segmented genome surrounded by the host cell-
derived lipid membrane. We focus on the two glycoproteins
embedded in the envelope membrane, hemagglutinin (HA) and
neuraminidase (NA), implicated respectively in cellular entry
and release of progeny viruses. Due to their surface exposure,
antigenicity of HA and NA categorizes influenza A viruses
into 17 currently known subtypes of HA (H1 to H17) and ten
of NA (N1 to N10). With segmented genome facilitating re-
assortment with different strains, the virus is able to emerge with
a new suite of segments and subtypes.[27,28] We hypothesized
that the chances of these antigenic shifts are modulated, and
foreshadowed, by incipient patterns in the sequences of the
circulating strains. And that these patterns may be distilled from
the IRD via appropriate statistical analyses.

Querying the IRD for all relatively recent and complete HA and
NA sequences, we ended up with 26,635, 7696 and 16,696 HA,
and 22,488, 7662 and 14,205 NA sequences for human, swine
and avian hosts respectively, collected within the 17 year period
between 1999 and 2016. The restriction to this time period arose
from the necessity to have a minimum number of sequences
each year for reliable statistical analysis. With the objective of
modeling the differences between host-specific strains at
any given point in time, we did not distinguish between anti-
genic subtypes. We expected that our classification algorithm to
automatically distinguish residue differences dictating sub-type
categorization if necessary. Additionally, we used sequential
numbering for referring to the residue positions, and did not
attempt to globally align the collected sequences. Not using
a standardized scheme (such as H3 numbering for HA, and
N2 numbering for NA) is driven by the idea that for a large
enough collection of sequences, the random variations at each
sequential position (which would be reduced by aligning to a
reference sequence in the standardized numbering process)
might be key to unraveling important predictive patterns.

A small excerpt of the HA sequences for human and swine
influenza between residues 275 and 295 (sequential numbering)
is shown in Table 1. For the majority of the residues, there are
variations within each species, as well as across. We asked if,
given a sufficiently large set of sequences collected within some
relatively short period of time (1 year), we can train a protein-
specific classifier that accurately models these subtle patterns
of variation to reliably recognize the host species. We found that
relatively simple decision trees are able to adequately model
the species specific patterns with high out-of-sample accuracy
reaching 95%-99% (See Fig. 5, plates A-D). For example, a
couple of rules encoded by the decision tree shown in plate B
of Fig. 5 are: if residue 78 is I or K, and residue 292 is N, D or
T, then the HA sequence is from a human host with less than
1% error. On the other hand, if the residue 78 is I or K, and
the residue 292 is K or E, and the residue 400 is V, then the
HA sequence is from a swine host with approximately 6% probability of error. The
tree encodes 5 such rules in total, each of which terminates
in a distinct leaf of the tree (the nodes at the bottom layer).

The structure of the inferred tree corresponds to the number
and complexity of the encoded decision rules, which vary with
the time period of collection of the sequences, the host species
involved, and the protein under study.

TABLE 1

Classification problem setup: Human & Swine Influenza A Viruses (HA Sequences, standard code for amino acids)

| Protein | Inferred Predictive Features (Features) |
|---------|----------------------------------------|
| HA      | 205, 207, 208                          |
| Swine   | 290, 291, 292                          |
| Human   | 240, 241, 242                          |
| site A  | 275, 276, 277                          |
| site B  | 278, 279, 280                          |
| site C  | 281, 284, 285                          |
| site D  | 288, 289, 290                          |
| offsite | 291, 292, 293                          |

| NA      | 204, 215, 219, 252, 343, 346, 372, 400 |
|---------|----------------------------------------|
| close to antigenic sites | 182, 309, 307, 417 |
| offsite | 48, 51, 97, 219, 307, 344 |

These decision trees are computed using unbiased recursive
partitioning[29] on sets of host-specific sequences drawn within
a period of 1 year. We measure model performance on the
training data with in-sample accuracy: which is the fraction of

TABLE 2

Inferred Predictive Features (Features numbers are listed in the sequential scheme)

| Protein | Inferred Predictive Residues (Features) | Minimal Feature Set |
|---------|----------------------------------------|---------------------|
| HA      | 157, 158, 159                          | 78, 137, 157, 187   |
| Swine   | 205, 207, 208                          | 207, 291, 241, 401  |
| Human   | 290, 291, 292                          | 77, 78, 137, 400, 401|
| site A  | 240, 241, 242                          | 402, 545            |
| site B  | 275, 276, 277                          | offsite             |
| site C  | 278, 279, 280                          |                     |
| site D  | 281, 284, 285                          |                     |
| offsite | 288, 289, 290                          |                     |
| NA      | 204, 215, 219, 252, 343, 346, 372, 400 |                     |
| close to antigenic sites | 182, 309, 307, 417 |
| offsite | 48, 51, 97, 219, 307, 344 |

[20] [21] [22] [23] [24] [25] [26] [27] [28] [29]
The key computational challenge here arises from the existence of many possible alternate choices of decision rule-sets that disambiguate the host species. This redundancy partially arises from dependencies among non-colocated residues required for correct assembly and function. Here we aim to curate the minimal set of residues that disambiguate the hosts (irrespective of the time period), and such dependencies imply that numerous equally accurate sets of rules exist. We solve this issue via iterative feature depletion: we construct a conditional inference tree, identify the most important residue (one that has maximum contribution in classification accuracy), delete that feature from the training algorithm, and re-run the tree inference. As we continue to iterate in this manner, in each step we compute the out-of-sample accuracy by applying the learned model on sequences from all other one year time periods. We stop if the out-of-sample accuracy falls below 90%, or if we run out of features. Carrying out this iterated deletion for all time-periods, we identify a sequence of decision trees, all of which are highly accurate models of host tropism, irrespective of the time period of analysis. Charting the number of times each residue appears as the most important feature, we end up with a small set that have maximal contribution in recognizing the target host. Once this set is identified, we train a random forest classifier with the residues as features, for each year. The in-sample accuracy achieved by these forests are then inverted to compute the year-specific jump risk. Our results for HA and NA, and for swine-human and avian-human jumps is shown in Fig. 1 plates A-B. The overall workflow of our algorithm is summarized in Fig. 5 plate E.

We can also restrict our algorithm to only access sequence data collected from just one country at a time, to construct a geospatial estimate of the time-varying jump risk (See Fig. 3). Due to the severe sparsity of sequences in the IRD for many countries (See Fig. 4 plate E), our geospatial predictions are relatively patchy, incomplete and suffers from widened confidence intervals. Nevertheless, we are able to pinpoint correctly the time and place of both the 2004 and 2009 events.

Discussion

To summarize our computational approach, we construct viral host recognizers (for human, swine and avian Influenza A) by using the primary structure of HA and NA proteins, to first identify a minimal set of residues that allow for good out-of-sample classification performance across the years, and then using this invariant minimal feature set to estimate the maximum in-sample classification accuracy for individual years. Finally, we interpret this time-varying accuracy as the inverse jump risk indicator for selected host-pairs.

Viral populations evolve continuously; thus an invariant minimal set of residues that disambiguate target hosts reflect the seats of fundamental differences in molecular structures driving host-specific infection and transmission processes. A known causal factor is the specificity of HA binding to avian-like α-2,6-sialic acid (SA) versus the mamalian-like α-2,3-SA receptors. Therefore, substitutions in and around the HA Receptor Binding Site (RBS) possibly could drive host specificity, and the HA minimal residue set we identified is consistent with this observation.

Structurally, the native HA is trimeric, and each monomer is comprised of a distal domain of globular shape (HA1), and a proximal stem anchoring into the viral lipid envelope (HA2). It is well-recognized that antigenic drift is driven by the accumulation of amino acid substitutions in HA epitopes that block SA interaction. The antigenic sites recognized by monoclonal antibodies with high neutralizing activity, tend to be similar across subtypes, and are generally categorized...
Fig. 3. Geo-spatial Emergence Prediction. Our algorithm may be used to geographically localize the emergence risk, by feeding it geographically stratified sequence data. The key challenge is the sparsity of sequences from around the world in the IRD, which degrades our accuracy. Nevertheless, as shown in columns A and B, we correctly localize both the 2004 H5N1 and the 2009 swine flu emergence. Note that we could not predict the risk elevation in Mexico prior to 2009 due to the extreme sparsity of collected sequences for S. America. Additionally, the algorithm also predicts correctly the risk elevation in the middle east in 2005 for the avian flu emergence, and the SE Asia in 2009 immediately after the swine flu outbreak.

Interestingly, not all residues in the minimal set have surface exposure. Nevertheless, these residues have been identified to have important roles in host specificity. HA-mediated membrane fusion in acidic environment is necessary for cellular entry, and human viruses appear to fuse at a lower pH than avian and swine counterparts. The residue in HA2 corresponding to sequential index 401 is near the tip of the fusion peptide, and substitutions in this region have been observed in experiments designed to characterize membrane fusion activity and virus stability. Substitutions in the second HA2 residue at sequential index 544 has also being implicated in maintenance of thermal stability and proper expression of HA in cells.
Given the minimal set of predictive features identified by our algorithm, we computed the variance at these residues for the host-specific strain ensembles, as the virus continues to evolve. As shown in plate A, we get a strong and significant positive correlation between human and avian specific strains, and a significant strongly negative correlation between avian and swine specific strains. The correlation between human and avian strains was also strongly negative, but not significant. Plates B and C show the mean Shannon divergence at the identified features for each pair of hosts. We see that for HA, the distance between human-swine and swine-avian roughly remains constant, whereas the distance between the swine and avian strains continues to diverge. Plate D shows number of sequences collected in the IRD over time, and plate E illustrates the geospatial imbalance in the database. The imbalance is more severe for swine and avian sequences. Importantly, we control for this imbalance, and we do not predict risk spikes only for places or times with most sequences.

\[ \rho = -0.48, p = 0.04 \]

We also computed the time-varying distance between host-pairs, measured as the average Shannon divergence at the residues of the minimal sets (for HA, in Fig. 4, plate B and for NA in Fig. 4, plate C). This distance for HA shows an intriguing pattern, it appears that the swine strains are equidistant on average from human and avian strains post 2004, whereas the swine and avian strains are strongly and significantly negatively correlated \( (\rho = -0.48, p = 0.04) \). The negative correlation between the human and avian strains, on the other hand, is not statistically significant. While not conclusive, these results are consistent with the suggestion that domestic pigs act as mixing vessels. Additionally, these strong correlations also support the thesis that the circulating strains interact continuously, and drive antigenic change.

The time-varying risk shown in Fig. 1 plates A-B illustrate that an impending jump can be predicted years in advance from observing the ever increasing risk elevation. The avian risk indicator compares favorably, with appropriate scaling, against the WHO report on H5N1 incidence since 2003 (See Fig. 1, plate C). While, we do not make a direct case that jump risk should translate to incidence rate, this close match is noteworthy.

We interpret these results suggest that the viral populations circulating in the respective hosts are continuously interacting, and driving each other’s molecular evolution. Without such continuous interaction, it is difficult to see how one would get a gradual increase instead of the risk spiking just before emergence. To investigate this claim further, we computed the mean standard deviation at the residues in the minimal feature set (for HA) over time (See Fig. 1, plate D and Fig. 4, plate A). The results show that with respect to this measure the human and swine strains are strongly and significantly positively correlated \( (\rho = 0.56, p = 0.01) \), and the swine and avian and swine strains are strongly and significantly negatively correlated \( (\rho = -0.48, p = 0.04) \). The negative correlation between the human and avian strains, on the other hand, is not statistically significant. While not conclusive, these results are consistent with the suggestion that domestic pigs act as mixing vessels.

Additionally, these strong correlations also support the thesis that the circulating strains interact continuously, and drive antigenic change.

In summary, the principal contribution of this study is an algorithmic approach to surveillance that exploits subtle patterns of sequence changes. These results fundamentally challenge how we think about bio-surveillance: we do not need to seek out the individual animals in which a chance re-assortment event gives rise to a pandemic strain, we can carry out random
Fig. 5. Examples of Inferred Conditional Inference Trees. Plates A-D illustrate conditional inference trees that recognize HA sequences pertaining to human vs swine (A,B) and human vs avian (C,D) for the respective years 2007, 2009, 2011, and 2013. The leaf nodes enumerate the majority class, along with the percentage class error. The colors of the node depict the relative mixture of the host species. The numbers in the non-leaf nodes denote the residue index (sequential numbering). These decision trees characterize the optimally inferred rules that allow one to decide the host species given the amino acid sequence. Note that the number of rules vary from tree to tree and over the years. The in-sample accuracy of these classifiers is over 93%, with out-sample accuracy greater than 90% for immediate future. Plate E enumerates a summarized sketch of the algorithm, along with the key steps. Steps II and IV are the computational bottlenecks.

Examples of Inferred Conditional Inference Trees.

| Year | Host Species | In-sample Accuracy | Out-sample Accuracy |
|------|--------------|--------------------|---------------------|
| 2007 | Human vs swine | >93% | >90% |
| Human vs avian | >93% | >90% |
| 2009 | Human vs swine | >93% | >90% |
| Human vs avian | >93% | >90% |
| 2011 | Human vs swine | >93% | >90% |
| Human vs avian | >93% | >90% |
| 2013 | Human vs swine | >93% | >90% |
| Human vs avian | >93% | >90% |

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