Ability to Replicate in the Cytoplasm Predicts Zoonotic Transmission of Livestock Viruses

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Understanding viral factors that promote cross-species transmission is important for evaluating the risk of zoonotic emergence. We constructed a database of viruses of domestic artiodactyls and examined the correlation between traits linked in the literature to cross-species transmission and the ability of viruses to infect humans. Among these traits—genomic material, genome segmentation, and replication without nuclear entry—the last is the strongest predictor of cross-species transmission. This finding highlights nuclear entry as a barrier to transmission and suggests that the ability to complete replication in the cytoplasm may prove to be a useful indicator of the threat of cross-species transmission.

Many emerging infections are caused by viruses that are transmitted between host species. Specific characteristics may pre-adapt some viral groups for cross-species transmission. If we can identify such characteristics, we can better prepare for future viral threats to humans, domestic animals, and wildlife that will emerge as interactions between potential host species change.

Previous studies have compared emerging human pathogens to nonemerging human pathogens and looked for characteristics typical of those considered to be emerging [1–3]. To ask which characteristics predict host jumps requires a different approach. Specifically, we must examine the pool of other hosts' pathogens that a target species regularly encounters. From this pool we can compare the characteristics of microbes that are able to infect the target host versus those that manifest no evidence of an ability to infect the target host. Molecular characteristics that facilitate cross-species transmission are likely to be substantially different between viruses, bacteria, and protozoa, because of large differences in the pathobiology of these different taxa.

Here, we focus on cross-species transmission of viral infections and examine the effects of 3 characteristics that are described in the literature as expected to affect the ability of a viral group to infect a novel host species: genome segmentation, genomic material, and site of replication. The ability to rapidly explore genetic state space is expected to increase the probability of a host jump, so we expect that viruses with RNA genomes will have a higher probability of jumping than viruses with DNA genomes [1, 4] and that viruses with segmented genomes will have a higher probability of jumping than viruses with nonsegmented genomes [4]. Complex interactions with a host’s cellular machinery, on the other hand, are expected to decrease the probability of a host jump, so we expect that viruses that are able to complete replication in the cytoplasm will have a higher probability of jumping than viruses requiring nuclear entry [5].

To examine the effects of these characteristics, we should choose a target species that will maximize the chance that viral infection due to cross-species transmission events will have been detected; the obvious choice is humans. Likewise, we should minimize differences in exposure of the target host to infectious virions produced by the source hosts. Humans have regular contact with all potentially infectious bodily fluids of domestic food animals; we thus ensure that the target species has contact with all viral groups infecting the source hosts by analyzing the pool of viral species known to infect sheep, goats, cattle, and pigs.

**Methods.** We constructed a database containing taxonomic and molecular data on known viruses of domestic artiodactyls. To determine which viruses to include in the database, we searched the primary literature for references documenting infection of these species with all recognized species in all viral genera known to infect mammals. For each viral species infecting sheep, goats, pigs, or cattle, we then searched the literature to determine whether human infections have been documented (see table A1 in appendix A, which appears only in the electronic version).
Our analyses indicate that viral species infecting domestic artiodactyls are more likely to infect humans if they
complete replication in the cytoplasm without nuclear entry. The observed effect of cytoplasmic replication on host-jumping ability is not surprising given the complex molecular pathways regulating nuclear entry. Viral species that are unable to complete replication in the cytoplasm require intracellular transport from the site of penetration, targeting of the nucleus through nuclear localization signals, and importation of genetic material, proteins, and/or whole virions through the nuclear pore complex [10]. The combination of molecular mechanisms governing this chain of events is likely to be highly host specific, because of strong selective pressure against admission of foreign particles into the nucleus. To date, discussion of barriers to viral replication has largely focused on receptors for cellular entry. The concentration on this aspect of the viral life cycle exists for 2 substantive reasons. First, the inability to enter a cell obviously precludes viral replication; second, several well-documented viral host jumps have been shown to occur after point mutations that modify interactions between viral particles and cellular receptors [11–13]. The effect of nuclear entry seen in our data set emphasizes that cellular entry, while a necessary step, is insufficient for completion of the viral life cycle.

The ability to produce genetic diversity is the factor most widely discussed as expected to increase viral host-jumping ability [1, 3–5, 14]. Although the observed effects of genomic mater-

| Rank | Model: \( \log(\lambda) = \beta_i + \beta_{\text{Seg}}X_{\text{Seg}} \) | \( \ln(\ell) \) | \( K \) | \( \Delta AIC_c \) | \( w_i \) | \( \beta_{\text{Seg}} \) | \( \beta_{\text{GM}} \) | \( \beta_{\text{SR}} \) | \( \beta_i \) |
|------|--------------------------------------------------|-----------------|-----|-----------------|-----|-----------------|-----|-----------------|-----|
| 1    | \( \beta_i + \beta_{\text{Seg}}X_{\text{Seg}} \)  | -76.4           | 2   | 0               | 0.53 | ...             | ... | 2.854           | -2.996 |
| 2    | \( \beta_i + \beta_{\text{Seg}}X_{\text{Seg}} + \beta_{\text{SR}}X_{\text{SR}} \) | -76.3           | 3   | 1.9             | 0.20 | 0.181           | ... | 2.811           | -3.000 |
| 3    | \( \beta_i + \beta_{\text{Seg}}X_{\text{Seg}} + \beta_{\text{GM}}X_{\text{GM}} + \beta_{\text{SR}}X_{\text{SR}} \) | -76.4           | 3   | 2.1             | 0.19 | ...             | 0.105 | 2.780           | -3.016 |
| 4    | \( \beta_i + \beta_{\text{Seg}}X_{\text{Seg}} + \beta_{\text{GM}}X_{\text{GM}} + \beta_{\text{SR}}X_{\text{SR}} \) | -76.3           | 4   | 4.0             | 0.07 | 0.172           | 0.058 | 2.772           | -3.011 |
| 5    | \( \beta_i + \beta_{\text{Seg}}X_{\text{Seg}} + \beta_{\text{GM}}X_{\text{GM}} \) | -83.9           | 2   | 14.9            | <0.01 | ...             | 1.578 | ...             | -1.847 |
| 6    | \( \beta_i + \beta_{\text{Seg}}X_{\text{Seg}} + \beta_{\text{GM}}X_{\text{GM}} \) | -83.7           | 3   | 16.7            | <0.01 | 0.272           | 1.500 | ...             | -1.846 |
| 7    | \( \beta_i + \beta_{\text{Seg}}X_{\text{Seg}} \) | -89.0           | 2   | 25.2            | <0.01 | 0.740           | ... | ...             | -0.814 |

**NOTE.** \( X_{\text{Seg}}, X_{\text{GM}}, \) and \( X_{\text{SR}} \) are variables indicating the molecular characteristics of a viral species (see Methods). \( \ln(\ell) \) is the log likelihood of the best-fit parameter combination for a given model. \( K \) is the no. of model parameters for a given model. \( AIC_c \) is the value of Akaike's information criterion with small sample size correction for each model; thus, \( \Delta AIC_c \) is the difference in \( AIC_c \) value between a given model and the best model (i.e., the model with the lowest \( AIC_c \) value). \( w_i \) is the Akaike weight of the model. \( \beta_{\text{Seg}}, \beta_{\text{GM}}, \) and \( \beta_{\text{SR}} \) are regression coefficients for genome segmentation, genomic material, and site of replication, respectively. \( \beta_i \) represents the estimated intercept for the best-fit parameter combination for each model.

**Table 1.** Comparison of logistic regression models.

**Figure 1.** Comparison of data and model predictions. Gray bars show the proportion of livestock viruses in each category that are known to infect humans. Dashed lines give the prediction of the best regression model (as determined by Akaike’s information criterion adjusted for small sample size), which includes site of replication \( X_{\text{SR}} \) as the only variable. Sample sizes are given in parentheses below each bar. The ability to complete replication within the cytoplasm is the single best predictor of whether livestock viruses infect humans. Error bars represent 95% exact binomial confidence intervals.
rial and segmentation were not statistically significant, our data do not necessarily contradict this expectation. The hypothesized effect of segmentation, in particular, may be obscured in our data set by a combination of the small number of viral species with segmented genomes and the absence of segmented DNA viruses. On the other hand, the lack of predictive power associated with genomic material and segmentation in our data set may indicate that consideration of these traits alone is insufficient to capture the potential to generate useful genetic diversity.

The degree to which the pool of viruses infecting domestic artiodactyls is typical of all potentially zoonotic viral species is uncertain. Other pools of viral species should be examined to determine the generality of our results. Similarly, further studies should examine whether the observed patterns hold for cross-species transmission of viruses to other target host species, including wildlife and domestic animals.

Given the rapid rates at which ecological relationships between species are changing as a result of anthropogenic landscape changes, global warming, and globalization of both human and animal populations, the development of indicators of the risk of cross-species pathogen transmission is an increasingly important goal. As humans, domestic animals, and wildlife are brought into contact with species from which they were formerly isolated, they inevitably encounter the pathogens that these species carry. The finding that the ability to complete replication in the cytoplasm is the best predictor of zoonotic transmission and that nearly half of domestic artiodactyl viruses that are able to complete replication in the cytoplasm can infect humans suggests that cytoplasmic replication will be a useful indicator of the ability of a newly encountered virus species to jump hosts, an essential prerequisite to epidemic or pandemic emergence [15]. It should be noted, however, that the present analysis focused exclusively on the ability to infect the target host, and the viral traits influencing this step in the emergence process may differ from those that predispose a virus to cause severe disease in a novel host as well as from those that facilitate transmission within a novel host species.

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