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Harnessing genomics to trace the path of a viral outbreak in African lions

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Predicting the emergence of novel infectious diseases requires an understanding of how pathogens infect and efficiently spread in alternative naïve hosts. A pathogen's ability to adapt to a new host (i.e. host shift) oftentimes is constrained by host phylogeny, due to limits in the molecular mechanisms available to overcome host-specific immune defences (Longdon et al., 2014). Some pathogens, such as RNA viruses, however, have a propensity to jump hosts due to rapid mutation rates. For example, canine distemper virus (CDV) infects a broad range of terrestrial carnivores, as well as noncarnivore species worldwide, with a host range that is distributed across 5 orders and 22 families (Beineke et al., 2015). In 1993–1994, a severe CDV outbreak infected multiple carnivore host species in Serengeti National Park, causing widespread mortality and the subsequent decline of the African lion (*Panthera leo*) population (Roelke-Parker et al., 1996). While previous studies established domestic dogs (*Canis lupus familiaris*) as the disease reservoir, the precise route of transmission to lions remained a mystery, and a number of wild carnivore species could have facilitated viral evolution and spread. In this issue of Molecular Ecology, Weckworth et al. (2020) used whole-genome viral sequences obtained from four carnivore species during the CDV outbreak, in combination with epidemiological data, to illuminate the pathway and evolutionary mechanisms leading to disease emergence in Serengeti lions.

KEYWORDS

canine distemper virus, disease transmission, epidemiology, morbilliviruses, pathogen spillover, phylodynamics

Canine distemper virus (CDV) is a highly contagious RNA virus of the genus *Morbillivirus*, which often causes fatal neurological disease. The proclivity of CDV to cross host species barriers raises concerns that the virus could pose a significant threat to both humans and threatened wildlife species. In 1993–1994, a CDV outbreak in the Serengeti ecosystem was particularly alarming to the conservation community, as it resulted in the death of approximately 30% of the lion population and also affected a number of additional carnivore species, including spotted hyaenas (*Crocuta crocuta*) and bat-eared foxes (*Otocyon megalotis*; Roelke-Parker et al., 1996). Domestic dogs were implicated as the primary reservoir and source of spillover (Cleaveland et al., 2000; Roelke-Parker et al., 1996; Viana et al., 2015), with evidence for CDV circulating within neighbouring dog populations as early as 1989 (Alexander & Appel, 1994). However, models based on serological data revealed asynchronous infection peaks in lions with transmission occurring even during vaccination campaigns targeting domestic dogs, suggesting other unknown host taxa may play a role in driving CDV epidemic dynamics in the Serengeti (Viana et al., 2015).

Revisiting this system over two decades later, Weckworth et al. (2020) dove deeper into the question of whether additional host species contributed to CDV transmission within the wildlife

community and ultimately to disease emergence in lions. The authors tackled this by the integrating complementary epidemiological and genomic approaches, including (1) reverse transcription quantitative PCR (RT-qPCR) CDV screening of 13 putative hosts, (2) serological examination of CDV exposure in hyaena over time and (3) genetic analyses of viral genomes from infected canid (domestic dogs, bat-eared fox) and noncanid (spotted hyaena, lion) species to refine the evolutionary and transmission history of CDV during the Serengeti outbreak. Through this multifaceted approach, Weckworth et al. (2020) found that although hyaena were not likely the primary source of the outbreak, the species played a critical role in CDV evolution and transmission to lions.

Pathogen phylogenies have been increasingly used to estimate the evolutionary history of infections and, when overlaid with associated metadata (e.g. host, time of sampling), can shed light on transmission dynamics in complex multihost disease systems (Figure 1). In this study, Weckworth et al. (2020) reconstructed the CDV phylogeny, using viral genome sequence and host data collected over a 4-year period spanning the outbreak, confirming previous findings by Nikolin et al. (2017) of two divergent co-circulating strains in the carnivore community; lion and hyaena CDV strains were more closely related to one another than to strains in wild and domestic canids. As hyaena and lion strains were interspersed within the noncanid clade, the authors concluded transmission between the two species was frequent, while ruling out the possibility of "spillback" of the noncanid strain into canids due to the monophyly of the two CDV lineages.

Using a "phylodynamic" framework based on coalescent theory and the viral mutation rate (Grenfell et al., 2004), Weckworth et al. (2020) estimated that canid and noncanid clades diverged ~ 10 months prior to the first case discovered in hyaenas. This finding is significant as it illustrates how the accumulation of mutations rapidly led to increased host tropism. This recent emergence of a new noncanid strain is consistent with the hypothesis of hyaena as an intermediate host facilitating transmission in lions. The timing of clinical observations also supports hyaena as an immediate source to lions, with cases observed ~ 2 months prior to first signs in lions. Furthermore, in the years preceding the outbreak, no CDV-specific antibodies were found in either noncanid species, whereas increasing seroprevalence was observed in domestic dogs (Roelke-Parker et al., 1996).

The branching patterns of a pathogen phylogeny may not allow conclusive determination of the direction of disease transmission or the primary source. Thus, Weckworth et al. (2020) used a discrete trait model (Lemey et al., 2009) to predict the host states of ancestral nodes and estimate the number of viral transitions (i.e. jumps) between any two host species along the CDV phylogeny. Their analysis predicted hyaena as the most probable ancestral host of all noncanid strains, strengthening the argument that the species was likely a direct source of infection to lions. In addition, frequent viral host jumps were observed among species within, but not between the canid versus noncanid groups. What this analysis was not able to determine, however, is the ancestral host of all CDV outbreak strains, which the authors deduce is domestic dog based on serological and demographic evidence (Cleaveland et al., 2000).

It is important to note that phylogenetic models do not always accurately reflect the complete pathogen transmission history, particularly with partial sampling of the host community, which

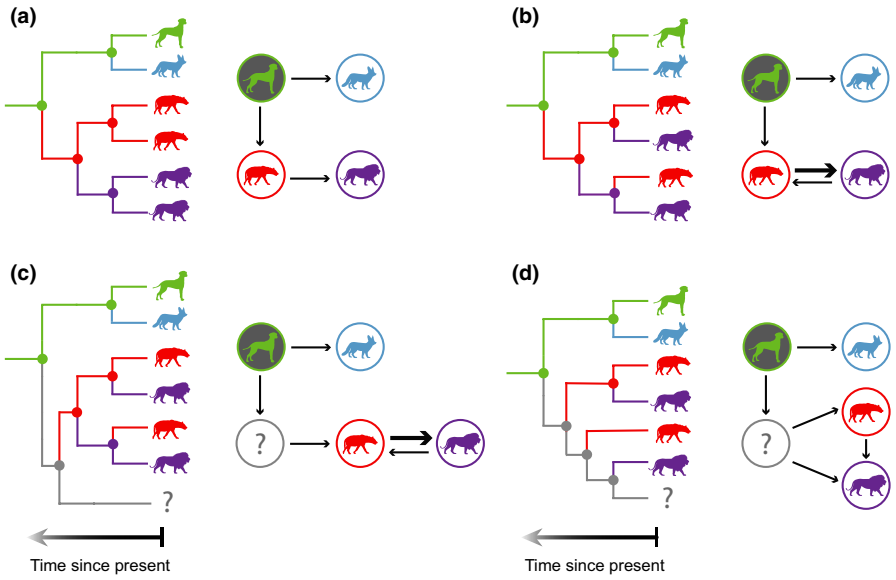

FIGURE 1 Pathogen phylogenies can be used to infer cross-species transmission dynamics in multihost communities. Discrete state models that predict ancestral host states back through time may shed light on the reservoir (grey shading) and intermediate hosts, as well as estimate the direction (arrows) and frequency of host jumps (arrow width). Genetic clustering of pathogens from the same host species (dog, fox, hyaena, lion) or group (canid, noncanid) suggests limited cross-species transmission (a), whereas topologically dispersed hosts indicate a greater degree of cross-species transmission (b). The role of additional hosts, however, could be overlooked, if unsampled (c, d) [Colour figure can be viewed at wileyonlinelibrary.com]

could distort inferences on transmission (Volz & Frost, 2013). Thus, the precise route from canid to noncanids could have involved unsampled "ghost" species (Figure 1c,d). In Weckworth et al. (2020), genomic sequencing was performed on archived samples primarily collected from individuals with outward clinical symptoms or as part of ongoing research activities; therefore, potential hosts playing a role in disease maintenance may have remained unsampled. For example, 1 out of 2 black-backed jackals (*Canis mesomelas*) screened was infected with CDV, but this species was not included in their phylogenetic analyses. In addition, the wild dog (*Lycaon pictus*) population was locally extirpated prior to the outbreak (Alexander & Appel, 1994), which precluded sampling, but suggests the species could have played a part in transmission among the carnivore community. While spotted hyaena are the most parsimonious source of CDV infections in Serengeti lions given the data available from the outbreak period, other canid sources remain a possibility.

The co-circulation of two host-specific strains during the Serengeti outbreak raises the question: Did the virus acquire adaptive mutations following spillover that enabled transmission within noncanids, but prevented spillback into the canid reservoir? Noncanid and canid CDV genomes from the outbreak were separated by 13 nucleotide substitutions, 7 of which were nonsynonymous and occurred in genes known to be associated with viral replication, immune evasion and host cell entry (Weckworth et al., 2020). This discovery suggests CDV underwent rapid evolution to overcome host barriers to infection. In all noncanids, rare mutations were observed in the haemagglutinin (H) transmembrane protein that binds specific host receptors and enables viral entry into host immune cells; these mutations were previously shown to enhance the ability of CDV to enter felid cells in vitro, and also to cause disease in other noncanid carnivores globally, such as the leopard *(Panthera pardus)*, raccoon *(Procyon lotor)* and American mink *(Neovison vison)*; Nikolin et al., 2017). Two mutations in the L gene, which encodes a polymerase required for viral replication, were also found in all noncanids except a single lion where the virus seemingly died out. However, this lion was from outside the core study area and could represent an unsampled lineage. Nonetheless, the absence of this L variant in an apparent dead-end lineage suggests the mutation could be important for enabling onward transmission within noncanids. Notably, 4 out of 7 of the nonsynonymous mutations were also shared with a CDV genome from South African hyaena (Loots et al., 2017), suggesting repeated parallel evolution of host tropism.

The study by Weckworth et al. (2020) highlights the importance of combining phylogenomic and epidemiological approaches to understand pathogen evolution and transmission in multihost communities. The authors present multiple lines of evidence that support hyaena as an important intermediary host, promoting rapid CDV evolution to noncanids and enabling disease emergence in lions. At the same time, they confirm hyaena were not the original source. In efforts to conserve the vulnerable African lion population, hyaena could serve as a possible sentinel species for detecting and mitigating rare CDV spillover events to lions. This study provides valuable insights, while warranting further study into the evolutionary mechanisms of morbillivirus emergence in naïve hosts worldwide.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no data sets were generated or analysed for this perspectives piece.

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REFERENCES

Alexander, A., & Appel, M. (1994). African wild dogs (Lycaon pictus) endangered by a canine distemper epizootic among domestic dogs near the Masai Mara National Reserve, Kenya. *Journal of Wildlife Diseases*, *30*(4), 481–485. https://doi.org/10.7589/0090-3558-30.4.481

Beineke, A., Baumgärtner, W., & Wohlsein, P. (2015). Cross-species transmission of canine distemper virus — an update. *One Health*, *1*, 49–59. https://doi.org/10.1016/j.onehlt.2015.09.002

Cleaveland, S., Appel, M. G. J., Chalmers, W. S. K., Chillingworth, C., Kaare, M., & Dye, C. (2000). Serological and demographic evidence for domestic dogs as a source of canine distemper virus infection for Serengeti wildlife. *Veterinary Microbiology*, *72*, 217–227. https://doi.org/10.1016/s0378-1135(99)00207-2

Grenfell, B. T., Pybus, O. G., Gog, J. R., Wood, J. L. N., Daly, J. M., Mumford, J. A., & Holmes, E. C. (2004). Unifying the epidemiological and evolutionary dynamics of pathogens. *Science*, *303*(5656), 327–332. https://doi.org/10.1126/science.1090727

Lemey, P., Rambaut, A., Drummond, A. J., & Suchard, M. A. (2009). Bayesian phylogeography finds its roots. *PLoS Computational Biology*, *5*(9), e1000520. https://doi.org/10.1371/journal.pcbi.1000520

Longdon, B., Brockhurst, M. A., Russell, C. A., Welch, J. J., & Jiggins, F. M. (2014). The evolution and genetics of virus host shifts. *PLoS Path*, *10*(11), e1004395. https://doi.org/10.1371/journal.ppat.1004395

Loots, A. K., Du Plessis, M., Dalton, D., Mitchell, E., & Venter, E. (2017). Genome sequences of three strains and two wild-type canine distemper virus strains from a recent disease outbreak in South Africa. *Genome Announcements*, *5*(27), e00603-17. https://doi.org/10.1128/genomeA.00603-17

Nikolin, V. M., Olarte-Castillo, X. A., Osterrieder, N., Hofer, H., Dubovi, E., Mazzoni, C. J., & Moehlman, P. D. (2017). Canine distemper virus in the Serengeti ecosystem: Molecular adaptation to different carnivore species. *Molecular Ecology*, *26*, 2111–2130. https://doi.org/10.1111/mec.13902

Roelke-Parker, M. E., Munson, L., Packer, C., Kock, R., Cleaveland, S., Carpenter, M., O'Brien, S. J., Pospischil, A., Hofmann-Lehmann, R., Lutz, H., Mwamengele, G. L. M., Mgasa, M. N., Machange, G. A., Summers, B. A., & Appel, M. J. G. (1996). A canine distemper virus epidemic in Serengeti lions (Panthera leo). *Nature*, *379*, 441–446. https://doi.org/10.1038/379441a0

Viana, M., Cleaveland, S., Matthiopoulos, J., Halliday, J. O., Packer, C., Craft, M. E., Hampson, K., Czupryna, A., Dobson, A. P., Dubovi, E. J., Ernest, E., Fyumagwa, R., Hoare, R., Hopcraft, J. G. C., Horton, D. L., Kaare, M. T., Kanellos, T., Lankester, F., Mentzel, C., … Lembo, T. (2015). Dynamics of a morbillivirus at the domestic – wildlife interface: Canine distemper virus in domestic dogs and lions. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(5), 1464–1469. https://doi.org/10.1073/pnas.1411623112

Volz, E. M., & Frost, S. D. W. (2013). Inferring the source of transmission with phylogenetic data. *PLoS Computational Biology*, *9*(12), e1003397. https://doi.org/10.1371/journal.pcbi.1003397

Weckworth, J. K., Davis, B. W., Dubovi, E., Fountain-Jones, N., Packer, C., Cleaveland, S., Craft, M. E., Eblate, E., Schwartz, M., Mills, L. S., &

Roelke-Parker, M. (2020). Cross-species transmission and evolutionary dynamics of canine distemper virus during a spillover in African lions of Serengeti National Park. *Molecular Ecology*, *29*, 4308–4321. https://doi.org/10.1111/mec.15449

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