# The One Past Health workshop: Connecting ancient DNA and zoonosis research

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# The One Past Health workshop: Connecting ancient DNA and zoonosis research

Sébastien Calvignac-Spencer[1)]* and Tobias L. Lenz[2)]*

The recent outbreaks of Ebola virus in West Africa [1] and Zika virus in South America [2] illustrate how global health security is regularly challenged by the emergence of zoonotic infectious agents. Predicting such events is largely hampered by our poor understanding of the conditions leading to zoonotic emergence. Deeper insights into the processes at play may therefore allow us to prevent zoonotic events or at least to detect them early enough to implement efficient control measures.

Unfortunately, the study of zoonotic emergence is facing significant hurdles. First, zoonotic events are rare. It is often necessary to collect and analyze massive numbers of samples to ensure their detection. Second, zoonotic events occur at complex human-domestic animal-wildlife interfaces. Understanding their drivers requires the implementation of holistic—and cost-intensive—approaches, for example *One Health* approaches [3]. Finally, we usually only learn of zoonotic events after—and sometimes long after—they happened. For example, SIVcpz was transmitted to humans in South-Eastern Cameroon in the early 20th century but the HIV-1 pandemic-to-come was not recognized before the early 1980s [4].

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Over the last two decades, we have circumvented these difficulties by using three strategies: 1) acquire massive amounts of information on selected contemporaneous systems and implement comparative approaches; 2) infer the origin in time and space of important zoonotic transmission events using nucleotide sequences; and 3) reconstruct the host/pathogen evolutionary arms race from their imprint at key genomic loci. It is only recently that the most intuitive approach of all, namely the direct collection of genomic information on past zoonotic events from archeological and museum specimens, has really become possible (Figure 1).

The One Past Health workshop, hosted by the Max Planck Institute for Evolutionary Biology (MPI-EB, Plön, Germany) and co-organized by the Robert Koch Institute (Berlin, Germany), aimed at offering a forum to scientists implementing these four approaches, under the premises that the recent democratization of the ancient DNA (aDNA) toolbox could unlock key questions in zoonosis research. More than 35 scientists from Belgium, Canada, Germany, Hungary, the UK, and the USA met from February 15–17, 2017 to present studies exploring any of these four directions and share their thoughts on aDNA developments. The meeting started with an aDNA-focused session which was followed by sessions dedicated to the other approaches.

## Ancient pathogen genomics

**Johannes Krause** (Max Planck Institute for the Science of Human History—MPI-SHH, Jena, Germany) introduced the general concepts and methods developed by paleogenomicists over the last decade, which witnessed an authentic scientific revolution with the rise of next generation sequencing (NGS). The coupling of hybridization capture with NGS was the decisive step that led to the first genomic studies focused on an ancient pathogen, the essentially tick-borne zoonotic agent *Yersinia pestis*, notably responsible for the medieval Black Death. The most recent aDNA findings identified *Y. pestis* in human remains from the Bronze Age. Their genomic make-up suggest these bacteria were not then efficiently vectorized by ticks, providing a striking illustration of the ability of aDNA approaches to catch zoonotic agent evolution red-handed.

**Alex Greenwood** (Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany) used much more recent samples, skin notches from koala museum specimens collected in the 19th and 20th centuries, to have a direct look at the evolution of the only retrovirus known to be currently undergoing endogenization, the koala retrovirus (KoRV). KoRV already infected koalas more than 100 years ago and has shown an astonishing genomic stability since then. The endogenization process probably started much earlier (20–50 ky ago), yet, most endogenous KoRV are very low frequency variants, suggesting it is still in an active invasion phase.

In rare cases, pathogen genetic material can be surprisingly well-preserved and even constitute a significant fraction of the total DNA found in ancient remains—as reported by **Verena Schuenemann** (University of Tübingen, Germany) for some ancient *M. leprae* infections. However, most samples will only contain minute amounts thereof, leading to complex metagenomic mixtures of sequences. The development of tools to explore such

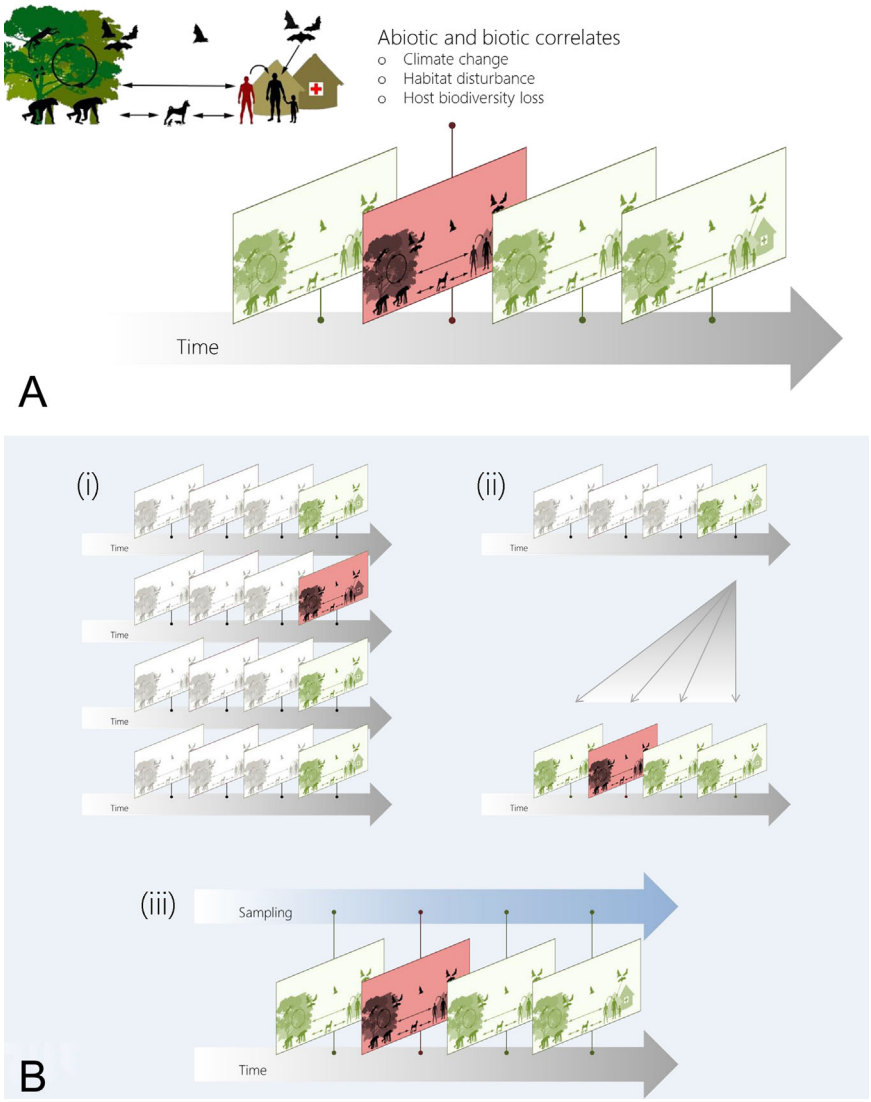


**Figure 1.** How to study zoonotic emergence? Panel **A** presents the usual theater of zoonotic emergence: complex human-wildlife interfaces in the tropics. In most cases, zoonotic events are not detected immediately. We would however need to understand the conditions that prevailed when the transmission took place (red slice) to identify potential predictors. In panel **B** we present three schematics representing the four strategies that can be used to reconstruct transmission events: (i) massive amounts of data are collected in replicate ecosystems with the hope that cross-species transmission will be detected and linked to particular local parameters (strategy 1 in the text); (ii) data obtained contemporaneously is used to infer what happened in the past: this is the approach used by sequence-based statistical inference methods as well as functional studies of the host/pathogen arms race (strategies 2 and 3 in the text); and (iii) heterochronous data (most frequently genetic/genomic, rarely proteomic) is acquired, opening a direct window on zoonotic emergence. Artwork credit: Kathrin Nowak.

complex datasets is thus a major stake for aDNA (and zoonosis) research. **Alexander Herbig** (MPI-SHH) presented MALT (Megan Alignment Tool), a fast read aligner and taxonomic assigner, which has been successfully implemented to analyze aDNA datasets. The availability of such tools marks the maturity of pathogen aDNA research, which has already clarified the evolution of some major zoonotic pathogens.

## Mass data collection and comparative approaches

Disease ecologists and medical molecular epidemiologists share a common interest for the processes shaping transmission patterns within and across species (including ours). They also rely on similar approaches, that involve the collection of a host of samples and comparative approaches. While introducing the general concepts of disease ecology, **Simone Sommer** (University of Ulm, Germany) particularly highlighted the fundamental notion of the "dilution effect," which posits that increasing host biodiversity leads to decreasing infectious agent transmission and prevalence. Since biodiversity decline is clearly linked to human disturbance, this hypothesis provides a link between human activities and increased zoonotic risk. Preliminary results from a disturbance gradient in Panama appear to support such a "dilution effect."

**Christian Drosten** (Charité, Berlin, Germany) provided a medical virologist's perspective on zoonosis research, exposing their unprecedented power to generate massive amounts of information on viral genetic diversity. How this genetic diversity translates into functional diversity and whether it can be used to predict zoonotic risk are the next big questions that need to be addressed. In this context, the isolation of novel viruses and/or reverse genetics is likely to hold the key to a better understanding of successful host-switch molecular determinants. Christian notably presented elegant functional analyses performed on recently discovered arthropod bunyaviruses. Information from these temperature gradient culture experiments revealed that the ancestor of these viruses was probably arthropod-specific (as opposed to vertebrate-specific or dual host tropic), that is, vertebrate-specific and dual-host lifestyles arose later in the evolution of this group.

Meta-analyses are also a powerful way to gather a critical mass of information to investigate cross-species transmission patterns. Using a comparative meta-analytic approach **Charles Nunn** (Duke University, Durham, USA) identified predictors of parasite sharing among primates, including phylogenetic relatedness, a larger geographic range, higher population density, and phenotypic similarity. He also reported on recent investigations on human parasite richness and presented preliminary analyses suggesting that it is pretty well predicted by our species phylogenetic placement. This

implies that the lifestyle changes that occurred since the Neolithic did not result in any net inflation or reduction of our parasite suite.

## Statistical inference

The development of molecular methods has fostered the parallel development of powerful statistical tools. This notably includes Bayesian evolutionary inference methods which are now firmly established as the tool of choice to decipher the origin in time and space, and the population size variations of pathogens. **Philippe Lemey** (University of Leuven, Belgium) highlighted that these space-and-time-aware methods can be enriched with generalized linear models (GLM) to assess the contribution of potential covariates. Such a phylogeographic GLM approach applied to a vast collection of Ebola virus genomes from the 2013–2016 West African outbreak showed that dispersal events were significantly less frequent between than within countries, and particularly so after the borders between the three most affected countries were closed. This model therefore suggests that border closure was effective in containing cross-border spread, although this did not curb outbreaks within countries.

A central question of zoonosis research is to estimate the number of host jumps, knowing we rarely observe them directly. Phylogenetic methods were developed to count host jumps from pathogen sequence data alone, including derivatives of Bayesian phylogeography. These methods rely on the strong assumption of an unbiased sampling effort across the putative source and sink host species. **Simon Frost** (University of Cambridge, UK) showed how biased sampling can result in an apparent reversal of the directionality of host jumps and sometimes lead to underestimating their number. Such artifacts can be corrected by incorporating external knowledge on directionality and sampling effort in likelihood-based models.

## Host/pathogen arms race

Zoonotic emergence is a multi-scale process. While influenced by macroscopic ecology, it is in the first place a matter of microscopic ecology, that is, cell-pathogen interactions. Both scales interact and ultimately determine zoonotic risk. The interactions of HIV and their simian progenitors, SIV, with the complex antiviral arsenal of their hosts have been studied in great detail. **Frank Kirchhoff** (Ulm University Medical Centre, Germany) provided a complete overview of how these viruses have frequently tailored their pocket genomes to counteract antiviral systems. A striking example concerns the key cellular transcription factor NFκB. Its activity is beneficial for the initial induction of viral transcription and HIV and SIV Nef proteins first potentiate it. Later on, however, HIV-2 and SIV Nef proteins down-modulate the expression of CD3, which curbs NFκB activity and prevents the NFκB-mediated expression of antiviral proteins. Surprisingly enough, the Nef protein of HIV-1 lost this second function. HIV-1 compensated this loss by using its late viral protein U (Vpu) to inhibit NFκB.

Epitope-presenting molecules of the major histocompatibility complex (MHC) are other key players that influence the evolution of HIV. **Jatin Arora** (MPI-EB) presented a computational screen of the entire HIV proteome, identifying a core set of disease-associated epitopes that appear to underlie the well-documented genetic associations between host MHC genes and HIV infection levels. This new approach provides an opportunity to interrogate pathogen genomes for relevant antigenic variation and will help to unravel the tight molecular interaction between hosts and their pathogens.

In contrast, the work from **Máté Manczinger** (University of Szeged, Hungary) investigated how pathogen selection can shape genetic variation of the host immune system. He reported that MHC gene pools of human populations appear to be adapted to the diversity of the populations' pathogen communities: Local MHC variants specifically target endemic pathogens in parasite-scarce environments while exhibiting exceptionally broad pathogen recognition capabilities in parasite-rich environments. The switch toward broader pathogen recognition appears to have occurred rapidly during human evolution, highlighting both the strong selective pressure posed by novel pathogen communities as well as the importance to comprehensively characterize host immune genetics when studying the molecular coevolution between specific host-pathogen systems.

## Future directions

This meeting was meant as a platform for a better integration of aDNA and zoonosis research. It led to fruitful discussions on conceptual and technical points of convergence. Of course, it was recognized that "classic" zoonosis research has much to offer to aDNA research on zoonosis. It offers fundamental insight into present-day pathogen biology, generates hypotheses about their evolution and produces key epidemiologic and genomic information, which simultaneously provides an essential background for evolutionary analyses and a rich catalog from which aDNA assays can be developed. Conversely, the potential of aDNA approaches in investigating zoonotic emergence was enthusiastically recognized by all participants. Ancient DNA approaches will help immensely in unveiling pathogen evolutionary timescales and characterizing the many unobserved past zoonotic events.

Our assembly also identified a number of promising research avenues. Viruses are overrepresented among emerging zoonotic agents [5] but it remains unclear which viruses will leave exploitable genetic traces in ancient samples. Viruses with a double-stranded DNA genome or a DNA genome intermediate (retroviruses) would be targets of choice but thus far the only robust results reported are limited to two genera of retroviruses and poxviruses. A systematic exploration of the question, most likely implementing hybridization capture approaches to compensate for the extreme dilution of viral genetic material, would be a welcome development. We also evoked medical archives and museum collections as a fantastic, and underexploited resource to study the processes at play during zoonotic emergence at a relatively high temporal resolution [6]. In addition, these collections might sometimes offer the opportunity to correct sampling bias from present-day sampling schemes. Finally, it was pinpointed that to turn aDNA-based evolutionary analyses into powerful predicting tools [7], the functional meaning of genomic variation should

also be investigated, which notably calls for more in cellulo functional studies.

We hope that this workshop will help foster the development of aDNA research on zoonosis, building on the broad set of approaches and skills already available in the zoonosis research community as a whole.

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