Opinion

Palaeoserology – teeth put into ancient plagues and pandemics

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Summary

Based on archived medical records and evolutionary modelling, a Coronavirus has been hypothesized as root and causative agent of the so-called 'Russian Flu' pandemic that surged in 1889–1890. In a *Correspondence* published in this volume of Microbial Biotechnology, Ramassy and colleagues try to support historical evidence by true experimental data using 'palaeoserology', a novel approach combining archaeology and modern immunological analysis. This *Opinion piece* tries to weigh arguments how strong such data may be, and where a refinement of methodology might be desirable before textbooks of medical history switch to call the 1890s pandemic 'Russian Corona'.

The suffering, death toll and disruption of societies caused by COVID-19 have sparked new interest in past pandemics and their causative agents. Infectious diseases in general and viral infections in particular have shaped human history ever since earliest hominid evolution (Zeberg and Pääbo, 2021), during major prehistoric events such as the Neolithic revolution (Serrano *et al*., 2021) and at key historic turning points (Marr and Calisher, 2003). However, lack of reliable medical records obscures identification of the respective pathogens. For the 1918–1919 'Spanish Flu', molecular methods have been used to reconstruct H1N1 Influenza A virus as culprit and deep analysis of its pathobiology (Morens and Taubenberger, 2018), uplifted by resurrection of live virus from viral RNA sequences recovered from flu victims buried and preserved under permafrost conditions. Besides this remarkable exception, lack of nucleic acid record generally hampers identification of past virus episodes, also considering inherent instability of RNA.

Likewise, exposure to infection shapes the individual antibody repertoire and leaves a unique pathogen-specific serological scar as kind of diary of one's former pathogen exposure. Persistence of pathogen-specific antibodies in convalescent plasma is the widely accepted concept behind any retrospective population-wide seroprevalence study (Metcalf *et al*., 2016). Of interest for the study of past epidemics, compared to RNA, proteins and hence antibodies are much more stable (Hendy, 2021) and may survive for long time *post mortem* (Larscheid *et al*., 2021) in niches such as dental pulp (Barbieri *et al*., 2017).

In a *Correspondence* published in this volume of *Microbial Biotechnology*, Ramassy *et al*. (2022) recovered antibodies from about 100 years old specimens of French recruits killed and buried early during WW I, in an experimental effort to provide serological evidence for the cause of the so-called 'Russian Flu', a severe acute respiratory syndrome pandemic that ravaged the world between 1889 and 1893 (Fig. 1). These ancient 'plasma donors' were old enough (> 25 years) to have likely been exposed to the 'Russian Flu' yet succumbed before emergence of the 'Spanish Flu'. The authors analysed antibodies from 29 specimens by semi-quantitative line probe assays, of which 5 (17%) turned positive for at least one Coronavirus, whereas only 1 (3%) for influenza.

What can be concluded from this 'palaeoserological' population profile? Do these findings support what archived clinical records (Brüssow and Brüssow, 2021) and, by coincidence, evolutionary clock modelling of human Coronavirus speciation (Vijgen *et al*., 2005) may suggest, namely, a beta-Coronavirus as root of the 1890s 'Russian Flu'? In partial fulfilment of Koch-Henle's postulates, i.e. induction of similar immune responses

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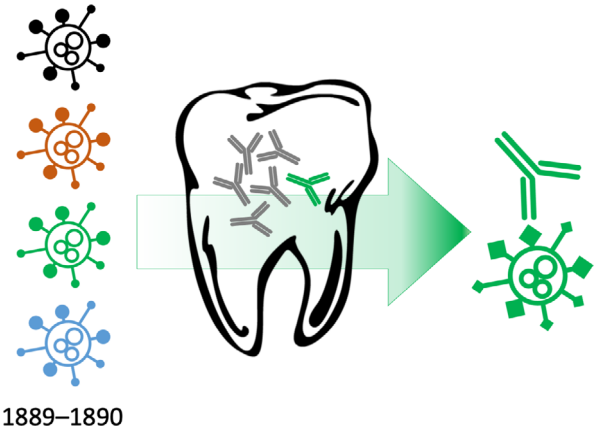

Fig. 1. Palaeoserology to unveil past pandemics. Alike forensic investigation is employed to provide scientific evidence during criminal investigation, palaeoserology is a diagnostic approach to support historical medical hypotheses. To that end, antibodies recovered from subjects exposed to a past pandemic are used as probe to assess serological cross-reactivity with known microbial pathogens. In a recent study, Ramassy *et al.* (2022) could hence isolate antibodies from the dental pulp of a small number of French soldiers who died in 1914 and had likely been exposed to the 1890–1893 'Russian Flu', showing that these antibodies differentially bind antigens of recent alpha- and beta-Coronaviruses, yet not Influenzaviruses. Should the 1890's pandemic hence not rather be called 'Russian Corona'?

shared amongst different subjects infected by the same virus?

Though highly suggestive, these data may need to be interpreted with some caution. Obviously, methodology needs refinement, and the performance of in-house assays (Oumarou Hama *et al.*, 2020) to be scrutinized using robust clinical diagnostic tests for cross-validation, including clear definition of limits of detection. Critical points to consider are the low available sample volume (pulp: around 20–50 μl) and physical limits inherent to antibody–antigen reactions. Human plasma contains about 15 g l^{-1} IgG (i.e. about 0.1 mM considering the M_w of IgG being about 150 kDa), whereas convalescent COVID-19 plasma – as a recent example for a severe Coronavirus infection – may contain relatively high levels of virus-specific antibodies (titres of, e.g. 1:1000) (De Giorgi *et al.*, 2021). The thus calculated specific antibody levels (0.1 Nm $\approx 10^8$ molecules μl^{-1}) may fall reasonably in the sensitivity of a high-performing ELISA ($\geq$ 1 pM $\approx 10^5$ molecules μl^{-1}) (Giljohann and Mirkin, 2009). Still somewhat in doubt, the author of this *Opinion piece* with his known vaccination, convalescence, and serostatus is willing to donate his next removed wisdom tooth to test whether (or not) from his dental pulp flu, COVID-19, and yellow fever-specific antibodies can be confirmed by the proposed methodology.

Two fundamental biological issues remain. Firstly, Coronavirus-specific plasma IgG is known to be transient and possibly waning in less than one year after natural infection (De Giorgi *et al.*, 2021). Thus, repeated exposure over several decades needs to be assumed for any 1914 war victim to remain his serological scar from the 1890's 'Russian Flu'. This is not unlikely, as pandemic 'Russian Flu' may have evolved into a seasonal common cold virus; in line with several yet successively damped waves of flu-like epidemics occurring between 1890 and 1914, commonly and possibly erroneously attributed to Influenzaviruses (Brüssow, 2021). More importantly, the choice of a proper antigen for serological detection remains critical. Any infectious agent that circulated in the past was likely different from those circulating today, at least as it concerns its antigenic protein structures. Notably, pandemics caused by rapidly mutating RNA viruses such as Influenza A viruses, or recent waves of SARS-CoV-2, are associated with a so-called 'antigenic drift' and 'variants of concern', respectively, with as hallmark a marked loss in cross-reacting antibodies (Simon-Loriere and Schwartz, 2022). How much can we hence trust a serological test that uses antigens as baits that are derived from current viruses to prey for antibodies elicited by 100 years old, possibly extinct, or at least mismatched antigenic structures? Others have tried to mitigate latter issue by introducing peptide sequence variation in their serological 'fingerprinting' of individual virus infection histories (Xu *et al.*, 2015). Reconstruction of evolutionary trajectories may finally resolve the inherent 'palaeodiagnostic' chicken-and-egg problem and help with an informed choice of appropriate 'palaeoantigens'. At the end, the 1890's pandemic may need to be renamed *'Russian Corona'*.

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Conflict of interest

The author has no conflict of interest to declare.

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