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Review

Equination (inoculation of horsepox): An early alternative to vaccination (inoculation of cowpox) and the potential role of horsepox virus in the origin of the smallpox vaccine

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ABSTRACT

For almost 150 years after Edward Jenner had published the "Inquiry" in 1798, it was generally assumed that the cowpox virus was the vaccine against smallpox. It was not until 1939 when it was shown that vaccinia, the smallpox vaccine virus, was serologically related but different from the cowpox virus. In the absence of a known natural host, vaccinia has been considered to be a laboratory virus that may have originated from mutational or recombinational events involving cowpox virus, variola viruses or some unknown ancestral Orthopoxvirus. A favorite candidate for a vaccinia ancestor has been the horsepox virus. Edward Jenner himself suspected that cowpox derived from horsepox and he also believed that "matter" obtained from either disease could be used as preventative of smallpox. During the 19th century, inoculation with cowpox (vaccination) was used in Europe alongside with inoculation with horsepox (equination) to prevent smallpox. Vaccine-manufacturing practices during the 19th century may have resulted in the use of virus mixtures, leading to different genetic modifications that resulted in present-day vaccinia strains. Horsepox, a disease previously reported only in Europe, has been disappearing on that continent since the beginning of the 20th century and now seems to have become extinct, although the virus perhaps remains circulating in an unknown reservoir. Genomic sequencing of a horsepox virus isolated in Mongolia in 1976 indicated that, while closely related to vaccinia, this horsepox virus contained additional, potentially ancestral sequences absent in vaccinia. Recent genetic analyses of extant vaccinia viruses have revealed that some strains contain ancestral horsepox virus genes or are phylogenetically related to horsepox virus. We have recently reported that a commercially produced smallpox vaccine, manufactured in the United States in 1902, is genetically highly similar to horsepox virus, providing a missing link in this 200-year-old mystery.

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1. Introduction

Vaccination was largely responsible for the eradication of smallpox declared by the World Health Organization in 1980 [1]. Most people are familiar with the work of Edward Jenner, who in 1798 published his famous work, often simply referred to as the "Inquiry", reporting that the inoculation of cowpox protected against smallpox [2]. Jenner called this new procedure "vaccine inoculation", to make reference to the cow (Lat. *vacca*), the proposed origin of the preventative against smallpox. Richard Dunning, a surgeon in Plymouth, coined the term vaccination in 1803 [3]. With the development of the germ theory of disease in the second half of the 19th century, a large number of specific microorganisms were identified as the etiological agents of different human and animal diseases. At the 7th International Congress of Medicine in London in 1881, to honor Jenner, Louis Pasteur proposed to generalize the term "vaccination" to include all protective immunization procedures against any infectious diseases [4,5].

A little-known fact is that Edward Jenner himself considered that the preventative against smallpox which is present in cowpox lesions, in fact derived from a disease of horses known as "grease" (horsepox). Early in the 19th century, European physicians conducted experiments that seemed to confirm the hypothesis that horsepox could protect against smallpox. Because of the relative rarity of cases of spontaneous cowpox, matter obtained from either cowpox or horsepox was interchangeably used in the protection against smallpox. Congruent with the use of the word vaccination, the inoculation from the horse (Lat. *equus*) was referred to as "equination" [6–8].

For much of the 19th century the true nature of the preventative against smallpox was discussed among the medical community, although it was usually assumed to be derived from cowpox [9]. It is important to appreciate that although the broader concept of transmissible agents of diseases was generally accepted by 19th century science, the medical community had to wait until the end of the century to have a clearer understanding of the role of microorganisms in the causation of disease. Thus, smallpox vaccination was developed and used in the 19th century based on enlightened empiricism. Viruses were only identified at the very end of the 19th century, based mainly on the criterion that their small size allowed them to pass through filters known to retain the smallest bacteria (filterable viruses).

The science of virology made considerable advances during the first half of the 20th century, including the ability to multiply viruses in embryonated eggs and tissue culture, thus allowing for a more detailed characterization of their biological, biochemical and immunological properties [10]. In 1939 Allan Watt Downie, a Professor of Bacteriology at the University of Liverpool, using serological techniques, demonstrated that the contemporary virus used for vaccination against smallpox, now referred to as vaccinia, was different from cowpox virus [11,12], reopening the scientific discussion about the true origin of vaccinia [13]. Since a natural animal host is not known, vaccinia is frequently referred to as a laboratory virus that could have originated from a still unidentified animal Orthopoxvirus ancestor. Based on the historical record, several investigators, especially the recently deceased Derrick Baxby (1940–2017), then a Lecturer in Medical Microbiology at the University of Liverpool, proposed that a presumed horsepox virus could be the long-sought ancestor of vaccinia [14–18]. Horsepox is a very rare disease that may have become extinct [19]. Fortunately, samples from a 1976 case of horsepox from Mongolia were used for genome sequencing, revealing that the horsepox virus is genetically related to vaccinia and might even be one of its ancestors [16]. Subsequently, other investigators have reported that contemporary vaccinia strains may in fact represent viruses derived from complex recombinational events between different strains of vaccinia that may have included a horsepox virus ancestor [20–22]. We recently reported that a smallpox vaccine commercially produced in the United States in 1902 is closely related to horsepox virus, confirming the hypothesis that at least some of the early smallpox vaccines were based on horsepox virus [23].

Here we review the historical data on the early use of horsepox to immunize against smallpox (equination), as well as relevant scientific information regarding vaccinia virus and its two most likely ancestors, cowpox and horsepox viruses, in an attempt to throw light on a 200-year-old mystery, the origin of vaccinia.

2. The historical record on equination

2.1. Jenner used both vaccination and equination to protect against smallpox

The "Inquiry", the most important work of Edward Jenner, was published in 1798 under the title of "An Inquiry into the causes and effects of the variolæ vaccinæ, a disease discovered in some of the western counties of England, particularly Gloucestershire, and known by the name of the cow pox" [2]. After documenting a number of people in which previous infection with cowpox protected against smallpox (or against inoculation of smallpox, an early prophylactic procedure also known as variolation), on 14 May 1796, Jenner inoculated an eight-year-old boy named James Phipps with matter obtained from a cowpox pustule on the hand of Sarah Nelmes, a dairy maid, who had been directly infected from a cow. That was the first and best-known experimental inoculation of cowpox done by Jenner. To confirm that the child was protected against smallpox, he was variolated six weeks later without showing any evidence of infection, providing the first experimental evidence that cowpox elicits protection against smallpox. At a time when the nature and properties of viruses were not known, Jenner had the intuition that the cause of cowpox was related to that of smallpox, and for this reason he invented the term of "variolæ vaccinæ", or "smallpox of the cow", to refer to cowpox.

Other authors have analyzed the strengths and weaknesses of the arguments advanced by Jenner to promote the use of cowpox as a preventative of smallpox [3,13,14,24–30] and those arguments will not be repeated here. We will focus instead on those aspects of the Inquiry that specifically relate to equination.

Jenner considered the question of the origin of "spontaneous" cases of cowpox and he made the observation that such cases were frequently observed in farms where the same workers took care of diseased horses and milked cows. He suggested that these farm workers were responsible for transferring the putative agent of cowpox from horses to cows. In fact, on the second page of the Inquiry [2], Jenner made the following comments: "*There is a disease to which the Horse, from his state of domestication, is frequently*

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