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Investigations on the historical origin and evolution of the smallpox vaccine

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SUMMARY

Smallpox, the disease that probably caused the highest death toll in human history, was declared eradicated in 1980 after an intensified campaign based on immunization with a vaccine initially developed by the British country doctor Edward Jenner in 1796. The classical story is that Jenner put to experimentation the folk story suggesting that milkmaids previously infected with a mild disease of cows (cowpox) were protected from smallpox. Vaccination was developed almost a hundred years before the germ theory of disease was formulated and, at that time, the nature of the immunizing agent was poorly understood. For more than 140 years it was widely accepted, in scientific and popular publications, that cowpox was the origin of the smallpox vaccine. However immunological research conducted in the 1930s revealed that cowpox and the smallpox vaccine (referred to as vaccinia) represented different viruses; an observation that many years later was confirmed by modern genomic analysis. Our collaborative group followed early clues suggesting that a related virus that infected horses (horsepox virus) could be the real origin of the smallpox vaccine. We initially analyzed the genomic sequence of a historic specimen of smallpox vaccine produced in Philadelphia in 1902 (Mulford 1902), providing the first scientific evidence of the suspected role of horsepox in the origin of the smallpox vaccine. More recently we reported that smallpox vaccines used during the 19th century in the United States, imported from Europe, included true horsepox viruses as well as vaccines representing intermediate strains between horsepox virus and modern vaccinia virus. We are now expanding our analysis to many other old smallpox vaccines, most of it still unpublished, and the available genomic sequence information suggests one or more evolutionary paths from a putative original smallpox vaccine based on the horsepox virus to the modern smallpox vaccine (vaccinia).

Key words: *Cowpox, horsepox, Jenner, smallpox, vaccine, vaccinia.*

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RESUMEN

La viruela, la enfermedad que probablemente causó el mayor número de muertes en la historia de la humanidad, fue declarada erradicada en 1980 después de una campaña intensificada que se basó en la inmunización con una vacuna inicialmente desarrollada por el médico británico Edward Jenner en 1796. La historia clásica es que Jenner puso en experimentación la creencia popular que sugería que las ordeñadoras previamente infectadas con una enfermedad benigna de las vacas (cowpox o viruela de las vacas) quedaban protegidas contra la viruela. La vacunación se desarrolló casi cien años antes de que se formulara la teoría microbiana de las enfermedades y

en ese momento la naturaleza del agente inmunizante era mal entendida. Durante más de 140 años fue aceptado ampliamente, en publicaciones científicas y populares, que el cowpox era el origen de la vacuna contra la viruela. Sin embargo, investigaciones inmunológicas llevada a cabo en la década de 1930 revelaron que el cowpox y la vacuna contra la viruela (conocida como vaccinia) representaban diferentes virus, una observación que muchos años más tarde fue confirmada por el análisis genómico moderno. Nuestro grupo colaborativo comenzó a seguir las pistas que sugerían que un virus relacionado de los caballos (horsepox) podría ser el origen real de la vacuna contra la viruela. Analizamos inicialmente la secuencia genómica de un espécimen histórico de la vacuna contra la viruela producido en Filadelfia en 1902 (Mulford 1902), la cual nos proporcionó la primera evidencia científica del presunto papel del virus del horsepox en el origen de la vacuna contra la viruela. Recientemente hemos reportado que vacunas contra las viruelas usadas en Estados Unidos en el siglo 19, importadas de Europa, incluían tanto el virus del horsepox como también vacunas que representan intermediarios entre el virus del horsepox y el moderno virus de vaccinia. Actualmente estamos ampliando nuestro análisis a muchas otras vacunas antiguas contra la viruela, la mayoría de lo cual todavía no hemos publicado y la información nos sugiere la existencia de uno o más caminos evolutivos desde la vacuna original contra la viruela, putativamente basada en el horsepox, hasta la vacuna moderna contra la viruela (vaccinia).

Palabras clave: Cowpox, horsepox, Jenner, viruela, vaccinia, vaccine.

INTRODUCTION

Smallpox was the disease that probably caused the highest death toll throughout human history. Its origin goes back many centuries and recent studies indicate that it could have been prevalent in Europe since the Viking era, more than a thousand years ago (1,2). Smallpox contributed to the population hecatomb that occurred in the Americas during the 16th century when the Spanish colonization began (3). In 18th-century Europe, smallpox killed nearly 400 000 people each year and it is estimated that, in the 20th century alone, smallpox killed between 300 and 500 million people all over the world.

VARIOLATION AND VACCINATION

An important early observation was that people who recovered from a smallpox attack never suffered the disease again, a concept that we now refer to as post-infection immunity. Smallpox was so feared that people in Asia began to practice the intentional and controlled inoculation of dried scabs from smallpox lesions in healthy individuals, to confer immunity against smallpox. According to Voltaire, the "inoculation for smallpox", later referred to as "variolation", was practiced in Asia since "time immemorial". Material for variolation was obtained from benign cases of smallpox and inoculations were performed after careful preparation of the subjects. Variolation was introduced in England in 1721 by Lady Mary Wortley Montagu, wife of the British Ambassador in Constantinople, where she became acquitted with the procedure. However, variolation was not a safe procedure, carrying up to 2 % chances of producing a fatal case of smallpox, or of starting new outbreaks of the disease. Despite all its associated dangers, variolation was widely adopted in Europe because of the fear of dying of naturally acquired smallpox (4).

It is in the theoretical context of variolation that vaccination was developed. During the 18th century, farmers in England observed that milkmaids who had become infected with a disease of cows known as cowpox became resistant to smallpox. The suspicion was that suffering the milder disease, which produces lesions similar to human smallpox in the teats of dairy cattle, could cross-protect against the most severe human disease.

In 1796, the British country doctor Edward Jenner decided to experimentally test the hypothesis that previous infection with cowpox prevents the development of smallpox. Jenner scarified the arm of an 8-year-old boy with the material of a cowpox lesion obtained from the hand of an infected milkmaid. Six weeks later, he scarified the arm of the boy again, this time with smallpox-derived material, basically conducting variolation. Because the boy did not develop any lesion at the site of variolation, Jenner became convinced of the "preventive power" of cowpox. These and other findings were gathered in a book published in 1798, usually referred to

as "the Inquiry" (5) (Figure 1). The procedure became known as inoculation of the cowpox or "vaccination", derived from "vacca", the Latin word for cow (6,7).

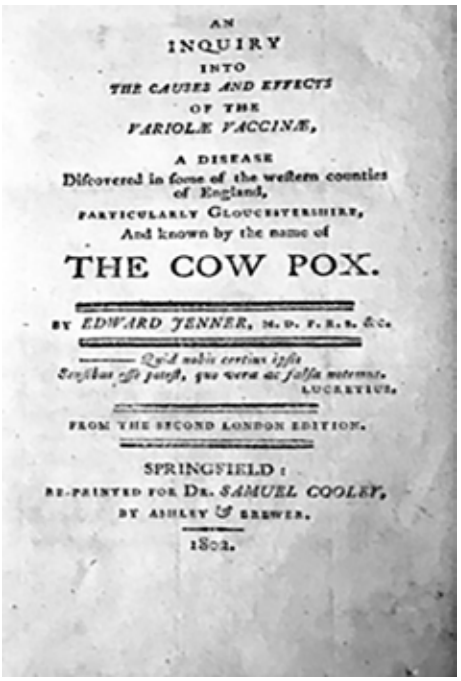
AN
INQUIRY
INTO
THE CAUSES AND EFFECTS
OF THE
VARIOLÆ VACCINÆ,
A DISEASE
Discovered in some of the western counties
of England,
PARTICULARLY GLOUCESTERSHIRE,
And known by the name of
THE COW POX.
BY EDWARD JENNER, M. D. F. R. S. &c.
——— Quid nobis certius ipsis
Sensibus esse potest, qui vera ac falsa notemus.
LUCRETIUS.
FROM THE SECOND LONDON EDITION.
SPRINGFIELD:
RE-PRINTED FOR DR. SAMUEL COOLEY,
BY ASHLEY & BREWER.
1802.

Figure 1. Title page of the first American edition of the Inquiry, published in 1802 in Springfield, Illinois, reprinted for Dr. Samuel Colley by Ashley & Brewer (J. Esparza collection).

The genius of Jenner was that he confirmed the validity of a folk tale with careful observation and experimentation, finding that vaccination was safe and effective. He also demonstrated that the cowpox could be serially transmitted from the arm of a vaccinated child to the arm of another child to be vaccinated, opening the possibility of implementing practical large-scale vaccination campaigns (7).

Jenner championed vaccination not just as a mean to achieve individual protection, but as a public health intervention, and Jennerian vaccination was rapidly introduced in Europe and elsewhere (8,9). The Inquiry was rapidly translated to many languages and Edward Jenner became a celebrity. His effigy was embodied in commemorative medals and prints that frequently recalled the supposed role that the cow played in the discovery (Figures 2 and 3).

An extraordinary example of the rapid spread of vaccination was the Royal Philanthropic Expedition of the Vaccine sent by King Charles IV of Spain to vaccinate the Crown's subjects in his territories around the world. Under the leadership of Dr. Francisco Javier de Balmis, the expedition departed from the Spanish port of La Coruña in 1803, just 5 years after Jenner's publication, with the vaccine initially carried by 22 children through arm-to-arm vaccination (10-12). The well-organized expedition, which was the first global immunization campaign, lasted until 1806 (Figure 4).

Figure 2. German medal honoring Jenner, used to promote vaccination (8). By Friedrich Wilhelm Loos, probably minted around 1838. The reverse shows seven children holding hands and dancing around a cow,the putative origin of the vaccine, which an angel adorns with a garland (J. Esparza collection).

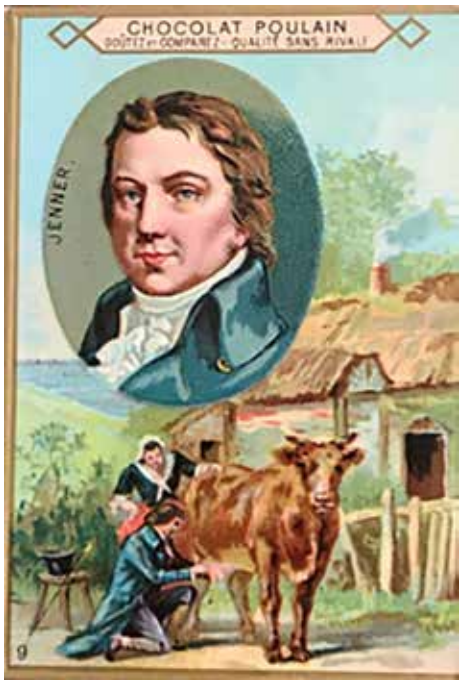

Figure 3. Advertisement card from a French chocolate factory honoring Jenner in a series on famous scientists (1900). The cow is a co-protagonist (J. Esparza collection).

TRATADO

HISTÓRICO Y PRÁCTICO

DE LA VACUNA,

Que contiene en compendio el origen y los resultados de las observaciones y experimentos sobre la vacuna, con un exámen imparcial de sus ventajas, y de las objeciones que se le han puesto, con todo lo demas que concierne á la práctica del nuevo modo de inocular.

POR J. L. MOREAU (DE LA SARTHE),

Profesor de Medicina, segundo Bibliotecario de la Escuela Médica de Paris, Catedrático de Higiene en el Liceo republicano, individuo de la Junta comisionada para observar la vacuna en el Louvre, de las Sociedades de Medicina, de la Medicinal de Emulacion, de la Filomática de los observadores del hombre, y Miembro correspondiente de la Sociedad Médica de Burdeos, de la de Emulacion de Abbeville y de Poitiers, y de la Sociedad de Ciencias y Artes de Maza.

TRADUCIDO

POR EL DR. D. FRANCISCO XAVIER DE BALMIS, Físico de Cámara de S. M., Honorario Cirujano de los Reales Exércitos, Profesor de Medicina, y Socio correspondal de la Real Academia Médica de Madrid.

SEGUNDA EDICION.

MADRID EN LA IMPRENTA REAL

AÑO DE 1804.

Figure 4. Title page of the book that members of the Balmis expedition left at each port visited, with instructions on how to practice vaccination. The book is a Spanish translation made by Balmis of the famous book by the French physician Jacques-Louis Moreau de la Sarthe (Historic and practical treatise of the vaccine). There are two Spanish editions of this book, 1803 and 1804 (J. Esparza collection).

For the first 80-90 years after Jenner discovered vaccination in 1796, the main procedure used to maintain and disseminate the smallpox vaccine was arm-to-arm vaccination, with the original material usually obtained from a cow suffering from cowpox. A major advance occurred after 1860 with the development of what was known as "animal vaccine", which referred to obtaining vaccine material from serial propagation in calves before used to vaccinate humans. This procedure allowed for the large scale production of smallpox vaccine and the establishment of the first vaccine industries (13). By the 1950s the extensive use of vaccination eliminated the disease in North America and Europe, but it continued prevalent in the developing world, although Chile, Venezuela, and Uruguay had eliminated the disease in 1954, 1956, and 1957, respectively. In 1967, the World Health Organization (WHO) launched the Intensified Smallpox Eradication Campaign that resulted in the eradication of the disease, declared in 1980, achieved thanks to the use of the vaccine first developed by Edward Jenner in 1796.

WHAT WAS THE ACTUAL MATERIAL USED BY JENNER TO VACCINATE?

The smallpox vaccine was developed almost a hundred years before the germ theory of the disease began to be formulated in the 1860s, and long before the nature of viruses became understood. Jenner and his contemporaries inoculated "material" that was capable of transmitting the disease, but they could not possibly understand the viral nature of the vaccine. Nevertheless, Jenner and other practitioners of smallpox vaccination during the 19th century showed an extraordinary capacity to infer complex scientific facts from carefully conducted clinical end epidemiological observations.

Since the time of Jenner, and for the following 143 years, it was widely accepted that the smallpox vaccine indeed originated from cowpox. That belief was challenged in 1939 when Allan Watt Downie (1901-1988), a Professor of Bacteriology

at the University of Liverpool, using serological techniques, demonstrated that the contemporary virus used for vaccination against smallpox, referred to as vaccinia, was different from the cowpox virus (14). This laboratory observation reopened an old debate about what was the true nature of the smallpox vaccine.

The nature of viruses began to be understood only at the end of the 19th century. And it was only in 1905 when the viral nature of the smallpox vaccine (a "filterable" virus) was recognized. Today we know that the Orthopoxvirus genus of the Poxviridae family includes several viruses that can elicit cross-immunity against subsequent infection with another orthopoxvirus, something that Jenner could not have known. Cowpox and variola viruses are both orthopoxviruses, but there are other viruses in that genus that could have also induced the observed cross-immunity.

Based on historical records several investigators, especially Derrick Baxby (1940-2017) then a Lecturer in Medical Microbiology at the University of Liverpool, proposed in 1981 that a presumed horsepox virus could be the long-sought ancestor of vaccinia (15). He concluded, however, that the mystery may never be satisfactorily solved.

WE FOCUSED OUR ATTENTION ON THE HORSEPOX VIRUS

After reading the book by Baxby more than twenty-five years ago, one of the co-authors of this article (JE) thought that Baxby was right and began to speculate how the mystery could be solved in the future (16), perhaps by comparing the molecular characteristics of vaccinia with those of the horsepox virus, although the existing DNA sequencing technology was not powerful enough at that time.

However, horsepox is a very rare disease that may have become extinct (17). Although horsepox has not been recognized in Europe since early in the 20th century (and never in the Americas), it was reported for the last time ever in 1976, in Mongolia. Fortunately, virus samples from Mongolian horses were available for genomic sequencing and in 2006 the results revealed that the horsepox virus is genetically related to vaccinia and might even represent the virus closest to an ancestor of the vaccinia lineage (18). Subsequently, other investigators have reported that contemporary vaccinia strains may represent viruses derived from complex recombinational events between different strains of vaccinia viruses that may have included a horsepox-like virus ancestor (19).

Important expertise, related to the solution of the mystery, was being perfected at the laboratory of another co-author of this article (AN) acquiring considerable knowledge on the use of Next Generation Sequencing (NGS) technology for the analysis of poxvirus genomes, particularly cowpox viruses (20).

The other co-author of this article (CRD) was independently working on the characterization of the Brazilian historical vaccine, vaccinia strain IOC, which was manufactured by Instituto Oswaldo Cruz in Rio de Janeiro, Brazil, and was the main smallpox vaccine used in Brazil until the late 1970s. The genome sequence of two IOC clones revealed their close genetic relatedness to clinical isolates of Cantagalo virus, a field strain of vaccinia virus that causes pustular disease in dairy cattle and milkers in Brazil, suggesting that Cantagalo virus probably represents an escapee of the smallpox vaccine once used in Brazil and the ancestor of the IOC vaccine (21-23).

The sequence analysis also revealed the relatedness of vaccinia strain IOC with horsepox virus that, together with Cantagalo virus, forms a new cluster within the vaccinia phylogeny. Interestingly, the relatedness of IOC and horsepox is also corroborated by the finding of inserts of horsepox DNA into some IOC genes, probably representing "molecular fossils" of its horsepox-like origins (23). Historical records account for the origin of the IOC strain probably being a French vaccine strain, the Beaugency Lymph, which was discovered near Paris in 1866 and was imported from France to Rio de Janeiro in 1887. Those results led to the speculation that the Beaugency Lymph may have been a horsepox virus or a vaccinia virus closely related to horsepox virus, but not cowpox virus as historically referred to as (24). In fact, the Beaugency lymph was distributed worldwide in the late 1800s, including several shipments to American vaccine farms, which may account for the genetic relatedness of the IOC/horsepox-virus

cluster with the American/Dryvax vaccine cluster within the vaccinia phylogeny (7,24).

Bringing together the expertise of the three groups, the time was right to form a collaboration and join efforts to try to sequence and, if possible, characterize the genomes of a collection of old smallpox vaccines that were in the possession of one of the co-authors of this article (JE).

We initially conducted an extensive review of the literature, documenting that Edward Jenner himself suspected that cowpox may have derived from horsepox and that he also believed that the "matter" obtained from either disease, cowpox or horsepox, could be used as preventative of smallpox. It was reported that during the 19th century, inoculation of cowpox (vaccination) was used in Europe alongside inoculation of horsepox (equination) to prevent smallpox (25). However, during the 20th century, the role of horsepox was somehow forgotten in favor of the most attractive theory of the cowpox infected milkmaid, with a story that was constantly repeated in textbooks and popular accounts, and the cow became, with Edward Jenner, the co-protagonist of that story.

AN EARLY AMERICAN SMALLPOX VACCINE BASED ON HORSEPOX

With that background, our collaborative group began to characterize the old smallpox vaccines from our collection, as well as several others that were provided to us by private collectors and medical museums.

Among the first vaccines that we analyzed was one manufactured in 1902 by Philadelphia's H.K. Mulford Company, which merged with Sharpe and Dohme in 1929 and eventually became Merck (Figure 5). Genomic and phylogenetic analysis of that vaccine revealed that the core genome of the virus in the vial of the Mulford 1902 vaccine has the highest degree of similarity (99.7 %) to horsepox virus (26) (Figure 6). Very interestingly, the deletions observed at the ends of the genome

Figure 5. Original wooden and glass containers that held capillaries containing the Mulford 1902 glycerinated vaccine (J. Esparza collection).

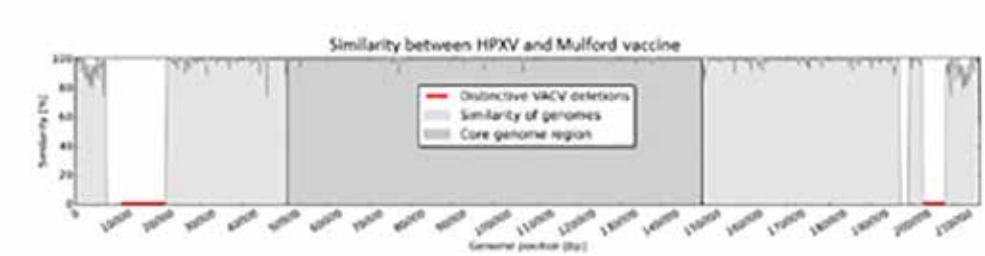

Figure 6. Similarity plots showing Mulford 1902 vaccine to its closest relative, horsepox virus (HPXV). The most similar sequences have been identified as the sequences spanning genes F9L to A24R according to the vaccinia Copenhagen annotation, highlighted in dark grey. The unique vaccinia deletion of 10.7 kb on the left and 5.5 kb on the right in the Mulford 1902 vaccinia, as compared with the horsepox virus sequence, are shown in red. From reference 26, reproduced with permission from the Massachusetts Medical Society).

of the Mulford 1902 smallpox vaccine strain are also found in the current vaccinia virus but not in cowpox or horsepox viruses. Therefore, the Mulford 1902 has hybrid features of horsepox (the central core region) and vaccinia viruses (the deletion at the genome ends). However, the Mulford 1902 vaccine clustered with horsepox virus, regardless of the method used to determine the phylogenetic relationships (Figure 7).

The results of our analysis of this 1902 smallpox vaccine provided the first scientific evidence of the suspected role of horsepox in the origin of the smallpox vaccine.

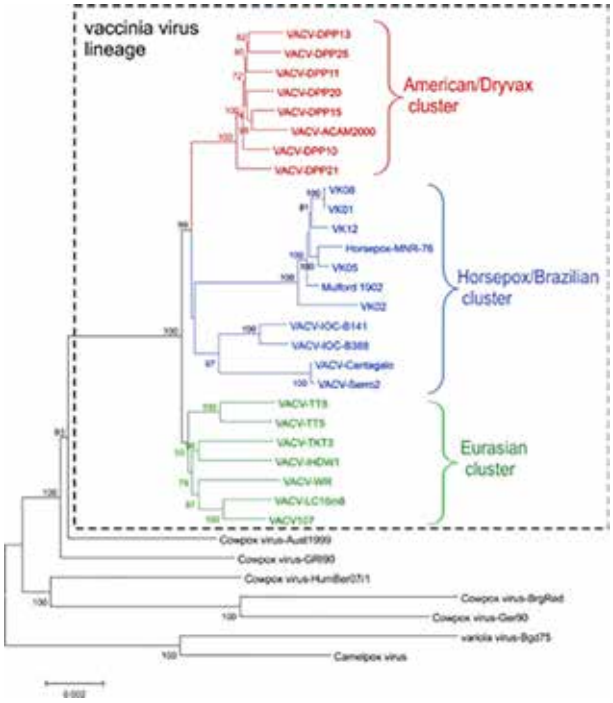

Figure 7. Phylogenetic tree of Mulford 1902, horsepox virus, representative vaccinia virus strains (VACV), and other Orthopoxviruses. The multialignment of the 33 Orthopoxvirus genomes was used to construct a neighbor-joining tree using MEGA 6 opting for kimura-2p model of substitution. Numbers indicate the percentage of 1 000 replicates of bootstrap support. In the horsepox/Brazilian cluster are included the Mulford 1902, which was the first known horsepox-based smallpox vaccine that we published in 2017 (26) and the VK vaccines that are 19th century American smallpox vaccines that we recently published (VK05 corresponds to a true horsepox virus vaccine) (28). Note that no smallpox vaccine maps in the same clade that variola or cowpox.

ADDITIONAL RESULTS CONFIRM A HORSEPOX ANCESTOR OF VACCINIA

A recent publication by another group (27) described the partial genomic sequence of five American smallpox vaccines from the mid-to-late 19th century. Phylogenetic analysis of these vaccines revealed that the vaccines are closely related to horsepox virus and the Mulford 1902 vaccine, confirming that horsepox-derived smallpox vaccines were used in the United States in the 19th century. Since neither cowpox nor horsepox are known to have existed in the Americas, these early vaccines were surely imported from Europe, indicating that horsepox-derived vaccines were also widely used in that continent.

Unfortunately, the above-mentioned article provided no information regarding the ends of the viral genomes, which are very important to understand the evolutionary relationship of the different smallpox vaccines. Consequently, our collaborative group reassembled the five published genomes and confirmed their close relationship to horsepox virus (28). Very interestingly, one of these five genomes (VK05) corresponds to a true horsepoxvirus, providing the strongest confirmation to date of the role of horsepox virus in the origin of the smallpox vaccine. Another genome was found to be the largest ever described genome in the vaccinia lineage. Our analysis showed that the five viral genomes from 19th-century American smallpox vaccines have different sizes and number of open reading frames (ORF), and interestingly, distinct structure in the left and right ends of the genome, representing different evolutionary intermediates between a hypothetical horsepox ancestor and the modern smallpox vaccines (vaccinia).

CONCLUSIONS AND ONGOING WORK

The available sequence information from old smallpox vaccines, most of it still unpublished, suggests one or more evolutionary paths from a putative original smallpox vaccine based on the horsepox virus to the modern smallpox vaccine (vaccinia).

The most interesting feature of this evolution

is the variable pattern of end deletions in the genomes of those old smallpox vaccines that could be related to gradual loss by ancestor horsepox-like viruses of genes related to host-range, immunogenicity, and reactogenicity. Host-range tropism may have changed when the horsepox based vaccines began to be passed in humans by arm-to-arm vaccination. In addition, the empirical selection of the best vaccines (more protective and with fewer side effects) may have also led to the selection of strains that have lost genes at the end of their genomes.

Orthopox viruses have low mutation rates and, after serial passage in culture, they show deletions, transversions, and duplications to a greater extent than point mutations. Similar events may have happened as a consequence of serial passage in humans and animals without proper control of passage number and the passage of different vaccine strains simultaneously. Animal vaccines started to be used in France in 1864 and the United States in 1870, and it is reasonable to assume that the mixing of different smallpox vaccines in cows resulted in a relatively high rate of homologous recombination (13).

Our collection of old smallpox vaccines spans specimens from the late 19th century to the 1970s, from different countries. These vaccines are currently being sequenced and analyzed and, hopefully, they will provide valuable information regarding the evolution of the smallpox vaccines. An interesting challenge is to understand how the horsepox-like conserved sequences located at the center of the genome, which codes for genes essential for virus replication, evolved to vaccinia-like sequences. Certainly, these were not long-term events since vaccinia and horsepox genomes share between 90 %-97 % similarities. Another, easier understand man-made evolutionary bottleneck, happened when in 1967 the World Health Organization undertook efforts to modernize and standardize the production of smallpox vaccines resulting in the selection of four main strains to be used worldwide in the Intensified Smallpox Eradication Campaign, which are representative of the contemporary vaccinia viruses. However, the establishment of the WHO era of smallpox vaccine strains does not account for the disappearance of horsepox-like vaccines by the end of the first half of the 20th century.

Therefore, mysteries still surround that period: why did horsepox disappear? How modern vaccinia virus appeared? Although Downie's studies in 1939 suggested that vaccinia and cowpox were different viruses, clearly indicating that the smallpox vaccine was not constituted of cowpox virus, we cannot rule out the possibility that Downie has analyzed horsepox virus (or a horsepox-based vaccine) instead of vaccinia. Based on the techniques available at that time, it was impossible to distinguish both viruses. The first detection of a true modern vaccinia virus has been recently made by our group with the, still unpublished, sequence of an American vaccine manufactured in 1930. This adds more wood to the fire making 1930-1940 a period of likely coexistence of horsepox-based virus and vaccinia virus.

The main results from our studies can be summarized as follows (29):

1. An analysis of the historical record, together with the genomic sequencing of old smallpox vaccines, supports the concept that horsepox and different horsepox-like viruses were used in the 19th century to vaccinate against smallpox.
2. We have no laboratory indication whatsoever of the use of cowpox as a smallpox vaccine.
3. Sequence information supports the hypothesis that the different horsepox-like viruses were the most likely ancestors of many old smallpox vaccines, evolving with the loss of genes at the end of their genomes.
4. An important shift happened around 1930-1940, when the smallpox vaccines changed from horsepox-like to vaccinia-like, with particular differences in the central conserved region of the genomes.

Although much remains to be done and learned about the origin and evolution of the smallpox vaccine, our initial work has attracted considerable attention (30), and already figures prominently in the most recent edition of one of the leading textbooks of virology (31), hopefully giving to the horse and the horsepox virus the right place on the history of the first vaccine ever developed.

ACKNOWLEDGMENTS

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REFERENCES

1. Mühlemann B, Vinner L, Margaryan A, Wilhelmson H, de la Fuente Castro C, Allentoft ME, et al. Diverse variola virus (smallpox) strains were widespread in northern Europe in the Viking Age. Science. 2020;369(6502): eaaw8977.
2. Alcamí A. Was smallpox a widespread mild disease? Science. 369:376-377.
3. Esparza J. La introducción de la viruela en América: epidemias en América Latina durante el siglo XVI. Tierra Firme (Caracas). 2000;72(XVIII):527-561.
4. Weiss RA, Esparza J. The prevention and eradication of smallpox: A commentary on Sloan (1755) "An account on Inoculation". Phil Trans Royal Soc B London. 2015;370:2014.0378.
5. Jenner E. An inquiry into the causes and effects of the variolæ vaccine, a disease discovered in some of the western counties of England, particularly Gloucestershire, and known by the name of the cowpox. London: Sampson Low; 1798.
6. Esparza J, Kerdel-Vegas, F. Bicentenario de la vacuna antivariólica de Jenner. Gac Méd Caracas. 1998;106:1-6.
7. Esparza J, Nitsche A, Damaso CR. Beyond the myths: Novel findings for old paradigms in the history of the smallpox vaccine. PLoS Path. 2018;14(7):e1007082.
8. Esparza J. Early vaccine advocacy: Medals honoring Edward Jenner issued during the 19th century. Vaccine. 2020;38:1450-1456.
9. Esparza J. Three different paths to introduce the smallpox vaccine in early 19th century United States. Vaccine. 2020;38:2741-2745.
10. Esparza J, Yepez Colmenares G. Viruela en la Venezuela Colonial: Epidemias, Variolización y Vacunación. In: Ramírez S, Valenciano L, Nájera R, Enjuanes L, editors. La Real Expedición Filantrópica de la Vacuna. Madrid: Biblioteca de Historia de América, Consejo Superior de Investigaciones Científicas; 2005.p.89-118.
11. Esparza J. Viral Epidemics in Latin America from the Sixteenth to the Nineteenth centuries and the early days of virology in the region. In: Ludert JE, Pujol FH, Arbiza J, editors. Human Virology in Latin America: from biology to control. Springer International Publishing AG. 2017.p.3-16.
12. Villamil Jiménez LC, Sotomayor Tribín H, Esparza J. Viruela en Colombia: de la Real Expedición Filantrópica al Parque de Vacunación. Bogotá: Sociedad Colombiana de Historia de la Medicina and Universidad de la Salle. 2020.
13. Esparza J, Lederman S, Nitsche A, Damaso CR. Early smallpox vaccine manufacturing in the United States: introduction of the "animal vaccine" in 1870, establishment of "vaccine farms", and the beginnings of the vaccine industry. Vaccine. 2020;38: 4773-4779.
14. Downie AW. The immunological relationships of the virus of spontaneous cowpox to vaccinia virus. Br J Exp Pathol. 1939:158-176.
15. Baxby D. Jener's smallpox vaccine. The riddle of vaccinia virus and its origin. London: Heinemann Educational Books; 1981.
16. Esparza J. Sobre el origen histórico del virus de la vacuna. In: Yepez Colmerares G, editor. Historia de la Salud en Venezuela. Caracas: Tropykos/CONICIT; 1998.p.31-50.
17. Esparza J. Has horsepox become extinct? Vet Rec. 2013;173:272-273.
18. Tulman ER, Delhon G, Alfonso CL, Lu Z, Zsak L, Sandybaev NT, et al. Genome of horsepox virus. J Virol. 2006;80:9244-9258.
19. Qin L, Favis N, Famulski J, Evans DH. Evolution of and evolutionary relationship between extant vaccinia virus strains. J Virol. 2015;89:1809-1824.

20. Dabrowski PW, Radonić A, Kurth A, Nitsche A. Genome-wide comparison of cowpox viruses reveals a new clade related to Variola virus. PLoS One. 2013; DOI: 10.1371/journal.pone.0079953

21. Damaso CR, Esposito JJ, Condit RC, Moussatché N. An emergent poxvirus from humans and cattle in Rio de Janeiro State: Cantagalo virus may derive from Brazilian smallpox vaccine. Virology. 2000;277:439-449.

22. Quixabeira-Santos JC, Medaglia ML, Pescador CA, Damaso CR. Animal movement and establishment of vaccinia virus Cantagalo strain in Amazon biome, Brazil. Emerg Infect Dis. 2011;17:726-729.

23. Medaglia ML, Moussatché N, Nitsche A, Dabrowski PW, Li Y, Damon IK, et al. Genomic analysis, phenotype, and virulence of the historical Brazilian smallpox vaccine strain IOC: Implications for the origins and evolutionary relationships of vaccinia virus. J Virol. 2015;89:11909-11925.

24. Damaso CR. Revisiting Jenner's mysteries, the role of the Beaugency lymph in the evolutionary path of ancient smallpox vaccines. Lancet Infect Dis. 2018; doi: 10.1016/S1473-3099(17)30445-0.

25. Esparza J, Schrick, Damaso, CR, Nitsche A. Equination (inoculation of the horsepox): an early alternative to vaccination (inoculation of the cowpox) in the prevention of smallpox and the potential role of the horsepox virus in the origin of the vaccinia virus. Vaccine. 2017;35:7222-7230.

26. Schrick L, Tausch SH, Dabrowski W, Damaso CR, Esparza J, Nitsche A. An early American smallpox vaccine based on horsepox. N Engl J Med. 2017;377:1491-1492.

27. Duggan AT, Klunk J, Porter AF, Dhody AN, Hicks R, Smith GL, et al. The origins and genomic diversity of American Civil War Era smallpox vaccine strains. Genome Biol. 2020; doi: 10.1186/s13059-020-02079-z.

28. Brinkmann A, Souza ARV, Esparza J, Nitsche A, Damaso CR. Re-assembly of 19th-century smallpox vaccine genomes reveals the contemporary use of horsepox and horsepox-related viruses in the United States. Genome Biology (in press).

29. Esparza J, Nitsche A, Damaso CR. The origin of the smallpox vaccine: Trying to set the record straight. Presented at the Symposium to commemorate the 40th anniversary of smallpox eradication. Annual Meeting of the American Society of Tropical Medicine and Hygiene. November 15, 2020.

30. Wu KJ. The mysterious origins of the smallpox vaccine. Smithsonian Magazine, September 4, 2018. https://www.smithsonianmag.con/science-nature/mysterious-origins-smallpox-vaccine-180970069/

31. Flint J, Racaniello VR, Rall GF, Hatziioannou T, Skalka AM. Principles of Virology, Chapter 1: Foundations. 5th edition. New York: Wiley; 2020.