Horses Used for Large-Scale Production of Immunoglobulins: An Inter-Species Approach

Abstract
This paper seeks a better understanding of the use of horses as serum producers for the pharmaceutical industry over the last 120 years. We discuss procedures and techniques applied in the production of conventional antivenoms, breaking the customary silence on the millions of horses that have been essential in the historical developments of scientific institutions, such as the Pasteur Institute, in France, and the Butantan Institute, in Brazil. By examining selected literature on the modern and contemporary history of antivenom therapy, we highlight prospects for the future, and in particular of the next-generation of antivenoms that eliminate the use of equines.

Introduction
The use of horse-derived plasma in large-scale production of immunoglobulins is currently adopted worldwide for treatment of various infectious diseases, as tetanus, rabies, botulism, and as the only treatment available for envenoming by several species of snakes, scorpions, and spiders. Beginning in 1894, in Europe and across the world, a revolutionary treatment to cure diphtheria, a widespread disease with high mortality rates that mainly affected children, wrote heterologous serotherapy into the history of modern medicine, through the contribution of renown scientists as von Behring, Kitasato, and Roux. In the hyper-immunisation process, horses are inoculated with several doses of a specific antigen (e.g. toxin) that induces an active immunological response in them by creating a level of antibodies high enough to neutralise the antigen lethal effects. When the horse serum presents high antibody levels (i.e. satisfied antibody titles), the animal is bled to extract the antibodies from the plasma.

Seeking for new angles in the history of serotherapy, from an integrative viewpoint of the humanities and the health sciences, we review the routine of the early equines used as living source of antibodies, in France of the late 19th century, and in Brazil of the 20th century. To the extent that today’s hyper-immune equine plasma production system remains so similar to the procedures adopted 120 years ago, early records may be used to disclose aspects of the past use of equines by the pharmaceutical industry and to prospect further considerations on current large-scale production system. Concerning its contemporary constraints, we also shed light on the limits and risks associated to the induced-adverse effects of heterologous immunoglobulins, prospecting trends and expectations regarding novel envenoming therapies.

It is not the use of animals in therapeutic practices that distinguishes modern medicine from the healing methods of antiquity or traditional practices. In fact, animals have long been associated with some sort of power in benefit of human health. Over the early decades of the 20th century, the Brazilian pharmaceutical industry offered a wide range of biological medicines. In 1920, the pharmaceutical repertory of the Vital Brazil Institute included 29 different types of horse therapeutic sera, and 31 opotherapics, which were pills composed of desiccated spleen, liver, ovary, mammary gland, red blood cells, and thyroid gland. In late 19th century, even in Europe, the medical industry worked closely with slaughterhouses to obtain such raw material. As a significant contribution to the history of animals in modern medicine, the challenges and trends resulting from intense and critical use of equines as living producers of antitoxins confront us with ethical concerns, as well as with the risks and limits of pharmaceutical products derived from horses. It is a history that has been largely ignored by those who, in other ways, have so fervently sought a better understanding of the multiple entanglements involving horses and human societies.

“At the cost of giving up all their blood...”
Jonathan Simon brings elucidative benchmark on the participation of equines on the Pasteur Institute, in Paris of the late 19th century. The early experimental use of horse-based antitoxin to treat ill children was carried out in early 1894, and consisted in the immunisation of horses with recovered bacteria from diphtheria infected children. These horses were given the names of children who had passed through the wards of the Hôpital des Enfants Malades. Stables became laboratories used for performing the novel routine of large-scale serum production. As experimental subject, each individual horse, as a manufacturing unit, was submitted to long-term and large-scale verification of several events aimed at techniques for maximising production of both the quantity and quality of the heterologous sera. In 1894, the national newspaper Le Figaro announced that: “if you go to the Pasteur Institute, at the foot of the garden, you can see around ten young cab horses between six and nine years old. They are comfortably installed in their stalls, marvellously well cared for and groomed, admirably fed, and can’t possibly miss their exhausting billets in Batignolles or Montrouge, to which fate had initially destined them” (7:4).

Considering the brutal reality of the horses explored in the streets of European cities, this scenario became partially true, thus comprising suitable publicity that was motivating generous donations in a national fund-raising campaign for the new treatment. In 1894, a retired Thoroughbred, Saliou, who had previously enjoyed success at the racetracks, was donated to the Institute’s diphtheria service. It was the first time that a Thoroughbred horse was admitted to the Institute stables. The symbolism of such initiative expressed a healthy philanthropic competition among the French bourgeoisie. Yet the fate of these horses was better captured by the literal words of the physiologist Emile Roux, who in the face of the urgency that led to increasing demand for serum and the fact of its slow production process, exclaimed, “our poor animals, even at the cost of giving up all their blood, would not be capable of furnishing one hundredth of the required quantity” (2:69–70).

In early 1895, the Pasteur Institute had about 138 horses, and a production of over 7,000 litters of blood. The true fortune of these animals would be better expressed later by the French writer, Marguerite Yourcenar: “the handsome steeds of the Garde Républicaine when they are old and broken, sent away to die, sometimes over a period lasting as long as two years, in a stall of the Institute Pasteur, where their sole diversion is to be bled (...), until
finally, void of blood, they crumble, equine tatters which are the victims to our progress in immunology. The men of the Garde themselves exclaim that they would much rather see them sent straight to the butcher shop.”

At that time, the new and vast field of antivenom therapy has also been developed by French scientists, as Calmette, Phisalix, and Bertrand, to face envenoming snakebites, a common threat to human survival in the tropical colonial territories. Concomitantly, studies developed by the Brazilian doctor, Vital Brazil, rectified Calmette’s principle of polyvalent serum, by demonstrating that an antivenom would only be effective against the same venom (or at least against the same snake genus) that had caused the envenoming.

Bleeding as an efficient matter
It is widely known that the first Brazilian public serotherapy institutions, such as the Butantan Institute, founded in 1901, were inspired in the technical expertise of the Pasteur Institute. Over the first decades of the 20th century, Vital Brazil was the head of the two major Brazilian serum manufacturers, the Butantan Institute, and the Vital Brazil Institute. In the Brazilian scientific literature, we found descriptions of experimental procedures which pushed the limits of horse’s physiology. In this sense, the early death (usually from liver disintegration, in consequence of amyloidosis), hyper-immunisation process performed by Vital Brazil for subcutaneous inoculation of pure scorpion venom into horses, is emblematic. Over a three-month period, twenty-four injections totalling an amount of venom equivalent to 1,512 scorpion bites was inoculated into one horse, an animal considered to be extremely sensitive to even small amounts of it. The bleeding was performed 11 days after. The horse’s reactions throughout the immunisation process were described in detail: “With each injection, [the horse] demonstrated intense reaction to pain, showing widespread trembling, breathlessness, nasal and tear hypersecretion, a rise in body temperature, intense sweating. Such symptoms lasted no more than 12 hours” (10:49).

Vital Brazil observed that horses do not always react in the same way to the induction of antitoxin production. So, rest may prove beneficial, while in others, after a few years of immunisation, the animal becomes a poor producer of antibodies, reacting little to the toxins. This matter was further addressed in a study that emphasised the hyper-immunisation as a concern of economic efficiency. Physiological constraints, as weight loss, unresponsiveness to increased feeding, and rest after immunisation, sudden death (usually from liver disintegration, in consequence of amyloidosis), cardio-hepatic-renal disorders caused by vast subcutaneous edemas, and fractures due to the accidental falls taken by weakened animals, indicated that the horse would be apt to be submitted to ‘total bleeding’. So, when the risk of animal’s death would represent consequent loss of a significant amount of blood for the serum production supply, total bleeding was performed to guarantee whole blood extraction. The method consisted of inoculating the animal with a saline solution that kept circulatory mechanics going, delaying haemorrhagic shock to allow the animal to survive until all the (diluted) blood could be withdrawn, in a procedure that could take as long as two days. This study also presents one of the rare records on deaths of horses used as serum producers by the Butantan Institute, during the years 1947 and 1948, (Figure 1).

From the 1970s, some refinements in the process of hyperimmunization guaranteed an increasing of horse’s survival. Nonetheless, the continuous process of injection of toxins imposes critical and ruthless consequences on the horse physiology and welfare which have been conveniently obscured by the industry of heterologous immunoglobulins. Currently, the lack of transparency of the Brazilian serum producer institutions can be verified from the absence of data available on the amount and origin of these animals, mortality rate, and destination for the horses unresponsive to the hyperimmunization, between other aspects related to their management. Is noteworthy that considerations on the welfare and ethical compliance
concerning humane use of horses in the production of antivenoms was only included in the ‘2016’s Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins’ of the World Health Organization.¹

The horse-derived serum and induced-adverse effects

In the early 1900s, adverse effects of products derived from horses, such as sera, were described,¹³ including anaphylaxis, serum sickness, and pyrogenic reactions, (Figure 2).

Anaphylactic reactions are known as early adverse reactions (up to 24 h) and can be either IgE-mediated or non-IgE-mediated. In the second case, a study demonstrated that 48% of snakebite victims that received horse-derived antivenoms developed anaphylaxis. The main problem of anaphylaxis is the severe anaphylaxis that can be life-threatening.¹⁴

Serum sickness is a late reaction (1–2 weeks after serum administration) caused by immune complexes (ICs) deposition in target tissues (e.g. blood vessels, glomeruli, and joints), mediating inflammation.¹⁵ Products derived from horse serum and administered as serotherapy are historically the most common cause of serum sickness. Although serum sickness is not life-threatening, it can have multi-organ involvement including acute renal failure.¹⁶

Pyrogenic reactions are neither rare. Characterized by contamination of the serum preparation by endotoxin or lipopolysaccharide (LPS), this response also contributes to the development of anaphylaxis since LPS can directly activate mast cells.¹⁷ Pyrogenic reactions were described in 27% of snakebite victims after receiving horse-derived serum.¹⁸

There are also uncertainty concerning to the heterologous serum efficacy. Most of the antibodies in horse-derived antivenoms are not directed towards venom components but are work against antigens that horses have encountered over the course of their lives (microorganisms, parasites, etc). Thus, most antivenoms contain about 70% of antibodies not directed against venom components, that is, they are not neutralizing antibodies.¹⁹ Underlying this significant problem is the question as to whether the remaining 30% of horse-derived antibodies targeting the key antigens are 100% neutralising molecules? For instance, the remaining 30% of specific antibodies cannot neutralise important toxins responsible for envenomings.²⁰

Unfortunately, Brazilian scientific study has tapped very little into these accounts of the induced-adverse effects of heterologous antibodies. Within Brazilian epidemiology and snakebite records, the occurrence of adverse effects is a matter that has been entirely ignored within most publications.²¹ A study demonstrated that the anti-crotalid antivenom produced by Butantan Institute, since
1901, keeping the same protocol. It also concluded the absolute ineffectiveness of the anti-crotalid antivenoms from Butantan Institute and Vital Brazil Institute antivenoms to immunorecognize crotamine.19 In the aftermath of the publication, the Butantan Institute improved its horse immunisation protocol, including only crotamine positive rattlesnake venoms. Yet, no study has yet been conducted to evaluate its change.

Finally, a structural aspect of heterologous antibodies pertained to the fact that horses are not a unit of production, but a biological system with a significantly diverse genetic and immune system which cannot provide a guarantee for uniform product quality at the end of the manufacturing process. Such constraint outlines continuous challenges faced by manufacturers to ensure the effectiveness of serum quality. The continuous increase in regulatory standards for biological medicines products has imposed real hindrances to ensure the supply of national (and global) demand for animal–derived immunoglobulins.1

The next-generation serum therapy: overcoming resistances in a current reality

Very limited efforts have been done to change the ancient use of horses as an antibody machine, especially in the field of antivenoms. Nevertheless, there were many progresses in antibody discovery technologies, antibody engineering approaches, and antibody manufacturing. The development of monoclonal antibodies (mAbs) was first described in 1975, with the discovery of the hybridoma technique. Although the hybridoma technique was described in 1975, only 10 years later, in 1985, the first monoclonal antibodies were released for therapeutic use in humans. In the last years, the pharmaceutical market with monoclonal antibodies had a remarkable growth.2 With 136 mAbs already marketed in 2021, there is an expectation of more than 200 in 2022. Indeed, mAbs (whether murine, chimeric, humanised or human) in the pharmaceutical market is a reality, being used as the most advanced therapies for the treatment of a diversity of diseases (e.g. cancer, allergy, autoimmune diseases, etc).

The current global therapeutic monoclonal antibody market was valued at approximately US of $150 billion in 2019 and is expected to generate revenue $300 billion by the end 2025.20 Even so, with many investments and mAbs available, many horse–derived sera are still used in therapy. Although we do not have any recombinant antivenom available, composed by a mixture of monoclonal antibodies, researchers from all over the world have been working in the field. There is a vast literature exploring basic research and pre–clinical tests with mAbs targeting venomous animals’ toxins. So, further steps are expected to renew focus on snakebite as a neglected tropical disease from research efforts within novel envenoming therapies.

The way forward to overcome structural limits of the conventional serotherapy can be uncovered into the contemporary developments of safer, healthy, and ethical therapy. The biopolitics of equine blood has led to the establishment of historical agreements between scientists, governments, industries, and societies. The Brazilian large-scale production of heterologous antibodies has been characterised by recurrent institutional and economic crisis, shortage, increasing the vulnerability of population in remote regions.21 Yet horses themselves have been part of the constellation of circumstances, expectations, and experiments which define both the scope and limits of the serotherapy over time. We present here a critical approach on the past and present production system of heterologous antivenoms, by examining structural harms specifically related to the centrality of the equine physiology in the serotherapy industry. The intense worldwide demand for hyper–immunized plasma has written a crude trajectory of horse exploitation which are still not entirely dimensioned.
Manuela Pucca holds a bachelor in Biomedicine, MS.c and Ph.D. in Immunology at the University of São Paulo, Brazil. She is a full professor at the Federal University of Roraima, and her research is focused on Immunotoxinology, on human recombinant antivenoms and bioprospection of venom-derived peptides. She leads clinical projects aiming to understand the severity of snakebite envenomings in Roraima.

Web: www.snakebiteroraima.com

Ana Lucia Camphora has degree in Psychology, Master’s in Psychosociology of Communities and Social Ecology, and PhD in Social Sciences. As independent scholar, investigates historical interlinkages between animals and human societies. Her current investigations are oriented to modern and contemporary equine cultures. The author thanks the Center for Contemporary Equine Studies for the support received for funding this research.

Email: alcamphora@gmail.com

Manuela Pucca holds a bachelor in Biomedicine, M.Sc and Ph.D. in Immunology at the University of São Paulo, Brazil. She is a full professor at the Federal University of Roraima, and her research is focused on Immunotoxicology, on human recombinant antivenoms and bioprospection of venom-derived peptides. She leads clinical projects aiming to understand the severity of snakebite envenomings in Roraima.

Web: www.snakebiteroraima.com