Clinical signs, diagnosis, and case reports of *Vaccinia virus* infections

ABSTRACT

Vaccinia virus is responsible for a zoonosis that usually affects cattle and human beings in Brazil. The initial clinical signs of the infection are focal red skin areas, fever, and general symptoms similar to those of a cold. Then, pustules and ulcerated lesions surrounded by edema and erythema follow, as well as local lymphadenopathy that can last for weeks. Cure and healing of the lesions occur over several weeks, leaving a typical scar in the skin of people and animals affected. The infection definitive diagnosis is made through morphological characterization of the virus by use of electron microscopy, followed by PCR for specific viral genes. Since 1963, circulating orthopoxviruses in infectious outbreaks in several regions of Brazil have been reported. Later, the etiological agent of those infections was characterized as samples of *Vaccinia virus*. In addition, the widespread use of those viruses in research laboratories and mass vaccination of militaries have contributed to increase the cases of those infections worldwide. Thus, several epidemiological and clinical studies are required, as well as studies of viral immunology, public health, and economic impact, because little is known about those *Vaccinia virus* outbreaks in Brazil.

Keywords: *Poxviridae* infections, virology, outbreaks, zoonoses, *Vaccinia virus*.

[Braz J Infect Dis 2010;14(2):129-134]

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INTRODUCTION

Poxviruses are epitheliotrophic viruses that have in common the tendency to cause skin lesions. They are complex viruses that replicate in cell cytoplasm of vertebrate and invertebrate hosts.[1]

Virions are constituted by a membrane, a nucleus, and lateral bodies, with or without an envelope. They have an ovoid or rectangular shape, and their genetic material is a linear double-strand DNA molecule, which can encode approximately 200 proteins (Figure 1).[1-2,4]

Of the known poxviruses, a genus stands out due to its medical importance and because it serves as a model for studying the *Orthopoxvirus* viral family, which comprises the smallpox virus and *Vaccinia virus* (VACV).[1-4]

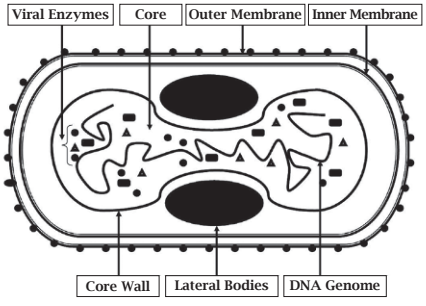
Smallpox was eradicated worldwide in 1980, after a large vaccination campaign, in which *Vaccinia virus* was used as a vaccinal vector, due to its high genetic similarity with the smallpox virus.[5]

Vaccinia virus is responsible for an important zoonotic disease affecting cattle and human beings in Brazil. The human zoonotic infections caused by poxviruses are relatively rare.[6]

However, zoonoses caused by poxviruses have occurred in regions far from their endemic areas, such as the outbreak of Monkeypox (MPXV) in central North-American states during May and June 2003, which was the first identified out of Africa.[7-9]

In Brazil, since 1960, innumerous outbreaks related to *Vaccinia virus* have been documented by several research groups in different regions. Based on molecular studies, some

Figure 1: Illustration of the morphology of the *Vaccinia virus*.



Submitted on: 01/20/2009
Approved on: 08/28/2009

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We declare no conflict of interest.

genetic variations have already been identified in the viruses isolated. An especially relevant finding was the isolation of two genetically different samples of *Vaccinia virus* from a single outbreak in the town of Guarani, in the Brazilian state of Minas Gerais.[10]

The number of human cases and new viruses isolated should increase because new epidemic foci have been reported in different areas. It has not been possible to determine whether *Vaccinia virus* infections are actually increasing or if reports have only recently begun.[11]

Episodes of *Vaccinia virus* infection in people who work at research laboratories have already been reported by the Center for Disease Control and Prevention (CDC), contributing to increase the number of infection cases.[12] The virus infection has occurred both in individuals previously vaccinated in childhood,[13-14] and in people never vaccinated

***Table 1. Clinical manifestations, viral diagnosis, and transmission of* Vaccinia virus**

	Clinical manifestations		***Viral diagnosis***		***Transmission***
Local	Start: focal red areas In a few days: pustules, edema and erythema in hands and forearms.[6,11,25] After approximately 12 days: ulcerated, necrotic and painful lesions.[6,11,25] A few days later, most lesions heal forming crusts. Approximately four weeks after lesion start: cure. Local lymphadenopathy that can last 20 days.[6,11,25] Secondary bacterial infections can occur in sites of original lesions.[26]	***Morphologic***	Inoculation of samples of lesions and crusts into allantochorionic membrane. Viral propagation in VERO cells and visualization of the viral particles through transmission electron microscopy.[11,23,27-28]	***Animals***	Transmission among animals occurs mainly through the milker's hands or mechanical milking equipment. Viral penetration occurs through preexisting lesions in cows' teats.[11,27,32]
Systemic	Fever, headache, muscle ache, nausea (occasionally) that begin eight days after the appearance of the lesions.[6,11,25]	***Molecular***	PCR of marking genes: thymidine kinase (TK), vaccinia virus growth factor (VGF), hemagglutinin (HA).[11,27,29] Polymorphism of the restriction profile of *ati* gene.[6,30] Real-time PCR of *ha* gene, by using SYBR Green.[31]	***Human beings***	The disease is transmitted from animals to humans through contact with the lesions in cow's teats.[33]

before.[15] Transmission usually occurs through accidental inoculation of *Vaccinia virus* through lesions in fingers and eyes, or through auto-inoculation.[13-16]

In addition to the cases of laboratory infection, cases from person-to-person contamination have been reported. Such cases are closely related to the vaccination of militaries against *Smallpoxvirus*.[17-18] Ten cases of infection by those viruses presumably transmitted through sexual contact have been reported in association with vaccination programs in the USA and Israel.[19-21]

Moreover, one case of vaccinia infection has been reported in a pregnant woman bitten by a dog previously vaccinated against rabies with a recombinant *Vaccinia virus*.[22]

Clinical signs, transmission, and viral diagnosis

Vaccinia virus infections can be characterized as occupational zoonotic infections because they occur in human beings who work directly with cattle, the milkers.[23] A fact of great relevance for public health is that physicians and other health care professionals have difficulty in diagnosing and managing these infections.[24]

The clinical manifestations, transmission, and viral diagnosis are shown in Table 1.

Vaccinia virus outbreaks in Brazil

Vaccinia virus outbreaks usually occur in small rural properties, with little infrastructure, and surrounded by woods. In most of these places, milking is performed manually, without the adoption of biosafety measures. These factors are believed to contribute to virus dissemination from cattle to milkers and vice-versa.[23]

This zoonosis seems to occur seasonally, mainly in the dry season, from July to September. Dry weather conditions seem to favor disease appearance and dissemination, since it contributes to dry cows' teats and milkers' hands, enabling the occurrence of lesions that cause the virus transmission from animals to men and vice-versa.[33]

Poxviruses isolated in Brazil

In South America a few studies on the isolation of *poxvirus* have been published since the eradication of smallpox. Some have reported outbreaks caused by *Parapoxvirus* in sheep and goats, and the virus isolation from wild or domestic animals.[11]

However indications that members of the *Orthopoxvirus* genus could be circulating actively in wild regions have been reported in Brazil since 1963.[28-29] In the 1960s and 1970s, the Brazilian govern carried out several campaigns of epidemiological surveillance in several rural areas of the country aiming at investigating the circulation of unknown viral agents and also the isolation of such new agents.[29]

Studies conducted from 1999 to 2007 in municipalities of Cantagalo, Cordeiro, Aperibé, Santo Antonio de Pádua, Cambuci, and Miracema have reported several cases of *Vaccinia virus* infection affecting both bovine animals and human beings.[34]

Several viruses isolated in Brazil in different regions after outbreaks of bovine smallpox are listed below. A summary containing viral samples isolated, hosts, and places of the outbreaks of *Vaccinia virus* infection are shown in Figure 2.

BeAn virus 58058: Belém Vaccinia virus

The *BeAN virus 58058* (BAV) was isolated in 1963 from the blood of a rodent of the *Oryzomis* genus in the tropical rain forest, in the region of Belém-do-Pará. After morphologic and molecular analyses, BAV was included in the *Poxviridae* family, considered a member of the *Orthopoxvirus* genus and a variant of *Vaccinia virus*, and denominated Belém *Vaccinia virus*.[28-29]

In addition, the type-A inclusion body (*ati*) gene could not be amplified through PCR, indicating its probable deletion. The IFN-IFN-α/βR gene was identified in the BAV genome, which showed a 99% identity with the B18R gene of the VACV-WR sample, a gene related to the evasion of the host immune system.[35]

Figure 2: Isolated viral samples, hosts, and places of the outbreaks of *Vaccinia virus* infection. *The SPAn232 virus was initially isolated in 1965 and classified as *Cotia virus* (LOPES *et al.* 1965).[23]

	1961	1693	1979	1993	1999		2000	2001	2003
Viral Sample	*Cotia Virus*	*BeAn virus 58058*	*SPAn232 Virus*	*Belo Horizonte Virus*	*Cantagalo Virus*	*Araçatuba Virus*	*Muriaé Virus*	*Guarani P1 virus Guarani P1 virus*	*Passatempo Virus*
Infected hosts	sentinel rats	Oryzomis sp Rodent	sentinel rats	Swiss mice	Cows and milkers	Cows and milkers	Cows and milkers	Cows and milkers	Cows and milkers
Place of occurrence	Cotia forest, São Paulo State	Tropical Rain Forests, Belém-do-Pará	Cotia forest, São Paulo State	Biological Sciences Institute of UFMG	Municipality of Cantagalo, Rio de Janeiro State	Araçatuba town, São Paulo State	Muriaé town, Minas Gerais State	Guarani town, Minas Gerais State	Passa-Tempo town, Minas Gerais State
References	LOPES *et al.*, 1965 [23]	DAFONSECA *et al.*, 1998 [15]	DA FONSECA *et al.*,2002 [16]	TRINDADE *et al.*, 2004 [29]	DAMASO *et al.*, 2000 [14]	TRINDADE *et al.*,2003 [11]	TRINDADE *et al.*, 2007b [19]	TRINDADE *et al.*, 2006 [10]	LEITE *et al.*, 2005 [13]

SPAn232 virus (SPAnv)

The *SPAn232 virus* (SPAnv) was initially isolated in 1961 from sentinel rats in the Cotia forest in the state of São Paulo.[36] The virus was re-isolated several times and suggested to be a recombinant of *Leporipoxvirus* and *Orthopoxvirus*.[37-39] It was originally grouped among the *Cotia virus*, but, after genetic analyses, it was considered a variant of *Vaccinia virus*. The *tk, vgf*, and *ati* genes were amplified and identified in genome of SPAnV, and showed a 99% similarity with correlate genes in VACV-WR .[29]

Cantagalo virus

The *Cantagalo virus* (CTGV) was isolated from cattle and milkers in 1999, during an exanthematic outbreak, in farms of the municipality of Cantagalo, southwestern region of the state of Rio de Janeiro. Morphologic and molecular evidence has confirmed that CTGV was a *Vaccinia virus* variant.[27,40]

After molecular analyses of the *ha* gene, a close relation of *Cantagalo virus* and VACV used in vaccination campaigns against smallpox in Brazil has been demonstrated.[27] It has been suggested that this sample escaped into the wild, establishing several cycles of transmission in one or more hosts, accumulating polymorphisms, reemerging, then, as *Cantagalo virus* in cattle and milkers.[27,40]

From October 2001 to July 2003, the Instituto Adolfo Lutz received 74 samples suggesting *Vaccinia virus* infection, from regions of the Brazilian states of São Paulo, Minas Gerais, and Goiás. Molecular analyses have categorized them as 99.9% similar to *Cantagalo virus*, differing only by a single nucleotide in position 616.[41]

Muriaé virus

In August 2000, an outbreak affecting cattle and milkers occurred in several farms of dairy cattle in the state of Minas Gerais. One virus was isolated and denominated *Muriaé virus* (MURV).[24]

During molecular characterization, when amplifying the *ha* gene, a deletion of 18 nucleotides was observed, allowing this virus introduction in the group of PSTV,[23] ARAV,[11] GP2V,[10] and CTGV.[27] Despite these similarities, *Muriaé virus* had unique characteristics that allowed its differentiation from other samples of VACV.[24]

Passatempo virus

The *Passatempo virus* (PSTV) was isolated and identified after an outbreak in 2003, in the towm of Passa-Tempo, Minas Gerais. During that outbreak, cows and milkers had lesions similar to those observed during other *Vaccinia virus* outbreaks in Brazil. When analyzing the blood of patients, antibodies against VACV–WR were identified.[23]

That *Vaccinia virus* variant, denominated *Passatempo virus*, has a deletion of 18 nucleotides in *ha* gene, which represents a genetic signature of some samples found in Brazil.[23]

Belo Horizonte virus

The *Belo Horizonte virus* (VBH) was isolated from an outbreak in mice of the facilities of the Biological Sciences Institute (ICB) of the Federal University of Minas Gerais (UFMG), in the state of Minas Gerais. The mice were brought from the University of Campinas, São Paulo, and seemed healthy upon their arrival at the UFMG. A few days later, some animals died and others showed skin lesions. The virus isolated from the clinical samples was a variant of *Vaccinia virus*, denominated *Belo Horizonte virus*.[42]

The origin of the *Belo Horizonte virus* remains unknown, since there is no research in the city of Campinas involving poxvirus. Those mice might have been contaminated by other animals of the nursery of ICB of UFMG, where some colonies of mice from other places are received. However, it is practically impossible to discover the virus actual origin. However, the ubiquitous circulation of different *Vaccinia virus* strains in Brazil, both from wild or veterinary origins, suggests that epidemiological studies are extremely important.[42]

Araçatuba virus

In 1999, in the city of Araçatuba, São Paulo, a virus was isolated after an exanthematic outbreak. The infection affected cattle and one milker, who developed approximately 10 lesions in his hands and arms. No similar episode had previously occurred in that farm.[11]

The isolated virus was a *Vaccinia virus* variant, and was called *Araçatuba virus* (ARAV). It had a deletion identical to that of *Cantagalo virus*.[11] It is worth emphasizing that similar genetic signatures occurred in the municipality of Cantagalo, located approximately 850 km to the east of Araçatuba, and in the town of Muriaé, 850 km to the north of Araçatuba, creating speculations about the origin of these viruses.[11]

Guarani virus

In October 2001, there was an outbreak in the town of Guarani, Minas Gerais, in the southeastern region of the country. An epidemiological study was carried out in the affected region and involved 72 properties. The study reported that 1,020 milking cows had lesions in their teats. Human cases of this disease were identified in 83% of the farms, and approximately 110 individuals were infected. In some farms, the milkers reported person-to-person transmission.[10]

For laboratory diagnosis and viral isolation, samples from the dry crusted lesions of two cows were collected. Each cow belonged to a different farm, approximately 10 km apart. Two viruses were isolated and denominated *Guarani P1 virus* (GP1V) and *Guarani P2 virus* (GP2V). Although isolated during the same outbreak and at the same time in neighboring farms, the GP1V and GP2V showed sufficient genetic divergences to be placed at different sites in the phy-

logenetic tree. While GP2V was placed with other samples of VACV isolated from bovine outbreaks of *Vaccinia virus* (ARAV, CTGV e PSTV), GP1V was placed with VACV-WR and VBH that are not associated with bovine outbreaks.[10]

These results indicate that there are genetically different populations of VACV circulating in the country and even in the same infectious outbreak. There are no conclusive studies on the actual origin of the Brazilian *Vaccinia virus*.

FINAL CONSIDERATIONS

Vaccinia virus infections are extremely relevant for public health and dairy economy in Brazil, although little is known about the virus flow in the wild and its natural hosts. It is difficult to define whether such infections have actually been increasing or if reporting has only recently started. In addition, health care professionals have difficulty in diagnosing and managing such infections.

Thus, implementation of educational strategies with health professionals and milkers who work in affected regions is required. For health care professionals, these strategies should be directed to the clinical identification and therapeutic management of infected patients. For milkers, the educational practices should emphasize biosafety aiming at preventing their contamination with the *Vaccinia virus* and reducing crossed infection in cattle. In addition, geoprocessing studies aiming at outlining the virus infectious flow are extremely important for the creation of health care strategies to decrease infection propagation, both among cattle and from cattle to human beings. Furthermore, several epidemiological and clinical studies are required, as well as studies of viral immunology, public health, and economic impact, because little is known about *Vaccinia virus* outbreaks in Brazil.

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