Antiviral Research 92 (2011) 150–163

Contents lists available at SciVerse ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Review

Zoonotic Brazilian Vaccinia virus: From field to therapy

Erna Geessien Kroon [a,*], Bruno Eduardo F. Mota [a], Jônatas Santos Abrahão [a], Flávio Guimarães da Fonseca [a,b], Giliane de Souza Trindade [a]

[a] Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG). Av. Antônio Carlos 6627, Belo Horizonte, MG 31270-901, Brazil
[b] Instituto René Rachou (IRR), Fundação Oswaldo Cruz. Av. Augusto de Lima 1715, Belo Horizonte, MG 30190-002, Brazil

ARTICLE INFO

Article history:
Received 6 June 2011
Revised 19 August 2011
Accepted 22 August 2011
Available online 27 August 2011

Keywords:
Orthopoxvirus
Vaccinia virus (VACV)
Bovine vaccinia (BV)
Antiviral drugs
Zoonosis
Occupational disease

ABSTRACT

Vaccinia virus (VACV), the prototype species of the *Orthopoxvirus* (OPV) genus, causes an occupational zoonotic disease in Brazil that is primarily associated with the handling of infected dairy cattle. Cattle and human outbreaks have been described in southeastern Brazil since 1999 and have now occurred in almost half of the territory. Phylogenetic studies have shown high levels of polymorphisms among isolated VACVs, which indicate the existence of at least two genetically divergent clades; this has also been proven in virulence assays in a mouse model system. In humans, VACV infection is characterized by skin lesions, primarily on the hands, accompanied by systemic symptoms such as fever, myalgia, headache and lymphadenopathy. In this review, we will discuss the virological, epidemiological, ecological and clinical aspects of VACV infection, its diagnosis and compounds that potentially could be used for the treatment of severe cases.

Contents

1. Introduction

In Brazil, bovine vaccinia (BV) is a disease that is caused by the Vaccinia virus (VACV). BV mainly affects milking cows and the dairy workers who have direct contact with those cows (Fig. 1C and D). BV is a typical *Orthopoxvirus* (OPV) infection that produces

* Corresponding author. Tel./fax: +55 31 3409 2755.
E-mail addresses: kroone@icb.ufmg.br, masc.egk@terra.com.br (E.G. Kroon).

0166-3542/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2011.08.018

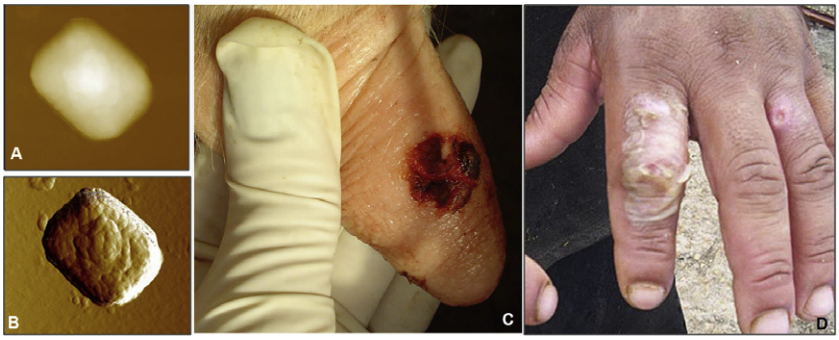

Fig. 1. (A) Phase air-dried and (B) high resolution height pictures of Br-VACV particles visualized using atomic force microscopy (AFM). (C) VACV exanthematic lesions in dairy cattle teats and (D) milker fingers during bovine vaccinia outbreaks.

lesions that evolve with the classic profile of a pox infection. Generally, dairy workers contract the infection through direct contact with animals during the milking process; therefore, the disease has been classified as an occupational zoonosis (Silva-Fernandes et al., 2009; Lobato et al., 2005). Since 1999, outbreaks in dairy cattle and humans have been described in Brazil. VACV has been detected in cattle, humans, rodents, monkeys and horses with and without clinical manifestations (Damaso et al., 2000; de Souza Trindade et al., 2003; Trindade et al., 2004; Silva-Fernandes et al., 2009; Brum et al., 2010; Abrahão et al., 2009a, 2010a).

The circulation of VACV in Brazil is significant in several regards. On one hand, the emergence of this infection poses a danger to public health and creates an economic burden for the dairy industry; on the other hand, the spread of this virus in natural environments and in domestic and wild animals presents an opportunity to expand the understanding of the natural history and evolutionary biology of VACV. Additionally, a clear understanding of the disease transmission risk factors and the extent of transmission to other barnyard animals and wild animals or to potential rodent reservoirs will provide data to support evidence-based prevention polices.

The increasing interest in poxvirus biology, in combination with a need for strategies to treat the infections caused by these viruses in humans and domestic animals, has led to the production of a wide array of compounds with potential anti-poxvirus activity. Most of these drugs are in the initial phases of study, and few of them are currently in human clinical trials or are commercially available. However, the market for anti-poxvirus therapeutics is growing rapidly, and new, safer, and more efficient compounds are discovered (or rediscovered) almost every day. The main goals to be achieved are the discovery of new compounds and the standardization of new regimens using one or more of these drugs to avoid undesirable outcomes, such as host toxicity and the emergence of drug-resistant viruses.

2. Vaccinia virus (VACV)

VACV was used in a worldwide vaccination campaign against smallpox in which several strains, such as Lister and New York City Board of Health (NYCBH), were distributed. In 1980, the World Health Organization (WHO) announced the eradication of smallpox. This incredible achievement was possible due to the widespread use of VACV as a vaccine to prevent smallpox transmission among humans (Fenner et al., 1988; Henderson, 2009).

Cowpox virus (CPXV) was the first virus to be used as a vaccine against smallpox. There are few reports that showed when VACV replaced CPXV as a smallpox vaccine. At the beginning of the 19th century, Edward Jenner reported the use of a virus isolated from an exanthematic disease in horses. This virus was designated "horsepoxvirus" and in 1817, it was distributed to the National Vaccine Establishment in Great Britain and, thereafter, to all parts of Europe to be used as a smallpox vaccine (Fenner et al., 1989). Recently, the genomic comparison of a virus named horsepox virus, which was isolated from diseased horses, revealed that the virus is closely related to other VACV strains (Tulman et al., 2006). The origin of VACV has never been elucidated and remains obscure to this day (Fenner et al., 1988).

Throughout the history of smallpox vaccination, several strains of VACV with different degrees of virulence were used around the world. With the creation of the Global Program for Smallpox Eradication, less virulent strains became the official WHO choice for mass vaccination (Henderson, 2009).

VACV is the prototype species of the *Orthopoxvirus* genus (family *Poxviridae*; subfamily *Chordopoxvirinae*), which also includes the Variola virus (VARV), Cowpox virus (CPXV), Monkeypox virus (MPXV), Ectromelia virus (ECTV), Camelpox virus (CMLV), Raccoonpox virus, Taterapox virus and Volepox virus (Moss, 2007).

The genome of VACV is composed of a single linear double-stranded DNA molecule of approximately 200 kilobase pairs (kbp) with a hairpin loop at each end. The genes located near the center of the genome generally encode conserved proteins related to essential functions *in vitro* such as DNA and RNA metabolism and structural proteins. The genes located near the termini of the genome encode proteins related to the host range and evasion of the host immune system and are non-essential for replication in culture cells. At both the right and left ends of the genome, there are repeated regions oriented in opposite directions, which, in some cases (with the exception of VARV), encode some genes. These regions are called inverted terminal repeats (ITRs). DNA sequencing of the central genes reveals that genes common to VACV, VARV, ECTV, CMLV and CPXV are greater than 90% identical (Moss, 2007).

Consistent with its complex structure, VACV encodes more than 200 genes that function in viral RNA synthesis, genome replication, virus assembly and host defense (Moss, 2007). The virion contains approximately 75 proteins, of which one third are enzymes involved in early mRNA synthesis, one quarter are membrane proteins, and the remainder are non-membrane structural proteins (Condit et al., 2006). Morphologically, the VACV virion is a brick-shaped structure with dimensions of $360 \times 270 \times 250$ nm (Fig. 1A and B). The mass of the mature virion was described to be 9.5 fg, and its principal components are protein, lipid and DNA, which account for 90%, 5% and 3.2% of the dry weight, respectively (Moss, 2007).

Like other poxviruses, VACV has a complex replication cycle (Fig. 2), which includes several infectious forms and a cytoplasmic site of replication, that is uncommon among vertebrate DNA

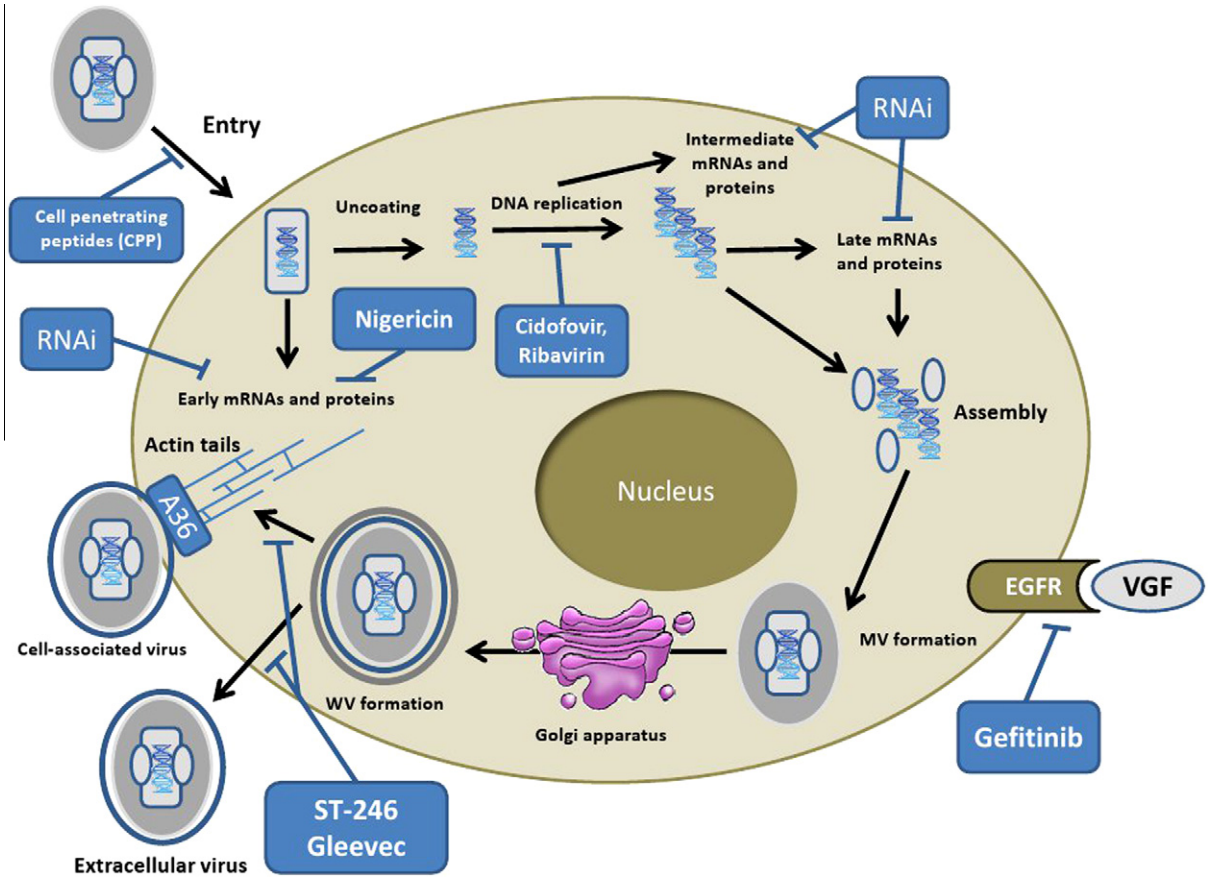

Fig. 2. A schematic view of the poxvirus replication cycle and the targets of antiviral therapy (blue boxes). The replication cycle begins with the entry of one of the two types of infectious particles, the mature virus (MV) or the extracellular virus (EV), by membrane fusion (only MV is shown). The viral DNA is then released into the cytoplasm, where it is replicated and then packaged into virus particles. The first infectious form discernible by electron microscopy is the mature virus (MV), which is released only when the cell lyses. A minor fraction of MVs are transported to the cell periphery and acquire two additional membranes from the Golgi apparatus to form the wrapped virus (WV). The WV then fuses its external membrane with the plasma membrane of the cell and is released into the extracellular medium, where it becomes a double membrane-bound extracellular virus (EV). Alternatively, the WV can fuse its external membrane with the plasma membrane but remain associated with the cell. When this occurs, the viral protein A36 is phosphorylated, inducing the nucleation of actin monomers to form actin tails beneath the virus particle. These actin tails propel the virus particle to neighboring cells, which provides a direct mechanism of cell-to-cell spreading. The particles associated with the actin filaments are referred to as cell-associated viruses. MV, mature virus; WV, wrapped virus; VGF, vaccinia growth factor; EGFR, epidermal growth factor receptor.

viruses (Moss, 2007). The stages of replication and morphogenesis afford opportunities for therapeutic intervention. The stages of VACV replication and the possible strategies for treatment are illustrated in Fig. 2.

3. The clinical aspects of Brazilian-VACV (Br-VACV) infections

The clinical presentation of OPV infections is characterized by a cutaneous manifestation with systemic or localized symptoms. The Br-VACV is associated with infections in humans, bovines and equines.

Lesions in BV-infected cattle are most frequently observed on the teats and udders of lactating cows. The lesions appear as bloody, painful wounds on large areas of the teat (Fig. 1C). The course of infection from the vesicle stage to complete healing takes 2–4 weeks (Simonetti et al., 2007; Schatzmayr et al., 2009; Megid et al., 2008; Lobato et al., 2005; Leite et al., 2005; de Souza Trindade et al., 2003), and the incubation period lasts from 1 to 7 days (Schatzmayr et al., 2009; Megid et al., 2008). Suckling calves can develop lesions on the face, and the disease is frequently clinically misdiagnosed as foot and mouth disease in these animals (Leite et al., 2005; Lobato et al., 2005). The neutralizing antibodies against VACV-WR are raised to titers of 1280 $PRNT_{50}$/ml in the convalescent phase (Simonetti et al., 2007; Costa et al., 2007; Lobato et al., 2005). The infection rates in cows range from 60% (Schatzmayr et al., 2009) to 100% (Silva-Fernandes, unpublished data; Donatele et al., 2007).

Moreover, some cows develop mastitis due to secondary infections (Leite et al., 2005; Lobato et al., 2005). Milk has been shown to be contaminated with Br-VACV (Abrahão et al., 2009c), and virus particles remained infectious after thermal treatment at 65 °C for 30 min in experimentally contaminated milk, which suggests that virus particles would also be present in solid curds and cheese whey (de Oliveira et al., 2010).

Horses present painful intradermal papules (2–3 mm in diameter) in the muzzle, between and surrounding the nares and on the internal and external surfaces of the lips. The lesions frequently showed a proliferative, verrucous aspect, with occasional exudation and bleeding upon manipulation. A similar pattern of lesions (with vesicles and eruptions) was observed on the internal surface of the lips, but without the presence of serous secretion. Small vesicles and papules were observed on the udder of a mare, and its foal showed lesions on the muzzle. The duration of the clinical symptoms was approximately 6–12 days, after which the animals progressively recovered. The overall duration of the disease in the herd was approximately 90 days (Brum et al., 2010).

In humans, VACV infection is characterized by the development of skin lesions, principally on the hands, that appear as itchy

nodular swellings (Silva-Fernandes et al., 2009; de Souza Trindade et al., 2007; Leite et al., 2005; Abrahão et al., 2009a). Other localizations of the lesions have been described as the nasal vestibule, periorbital and intraorbital areas (Silva et al., 2008; Megid et al., 2008), face (Simonetti et al., 2007), legs, arms, scrotum (Schatzmayr et al., 2000), mouth (Megid et al., 2008) and vulva (Schatzmayr et al., 2009). After a few days, the nodular swellings become papules, and local edema appears (Fig. 1D). The lesions evolve into umbilicated pustules surrounded by a strong inflammatory response. Approximately 12 days after the initial appearance of the lesions, they turn into necrotic and painful ulcers; after a short period of time, all of the lesions develop into scabs, most of which slough off. Exanthema is coincident with the development of peripheral lymphangitis and lymphadenopathy. The disease progression is accompanied by systemic influenza-like symptoms including headache, myalgia, and fever. The systemic symptoms are present for approximately 2–5 days (Megid et al., 2008) within days 3 through 20 following the initial onset of symptoms (Lobato et al., 2005; Silva-Fernandes et al., 2009; Leite et al., 2005; de Souza Trindade et al., 2007; Abrahão et al., 2009a). Other symptoms include anorexia, dehydration, arthralgia, nausea and sudoresis (Silva et al., 2008; Nagasse-Sugahara et al., 2004; Medeiros Silva et al., 2010). The exact incubation period of the infection is difficult to determine, but it has been described as 3–5 days (Schatzmayr et al., 2009). The whole progression of the disease takes 4 weeks. Secondary bacterial infections have also been described (Schatzmayr et al., 2009). Estimating the number of BV human cases in the last decade is virtually impossible because misdiagnosis and underreporting are frequent. However, undoubtedly, the most frequently BV-affected professionals are milkers and other rural workers.

4. Diagnosis

The diagnosis of VACV is usually performed according to the methods described for other OPV infections (Kulesh et al., 2004; Lobato et al., 2005; Trindade et al., 2006; Saijo et al., 2008; Vestergaard et al., 2008; Strenger et al., 2009). VACV diagnosis involves clinical, serological, virological, microscopic and molecular techniques (Lobato et al., 2005; Trindade et al., 2006; Trindade et al., 2009). VACV has the potential to cause local vesicular-pustular lesions, which are associated with fever, lymphadenopathy, and other symptoms (Silva-Fernandes et al., 2009). Due to the robust humoral immune response caused by VACV, antibodies can be detected with ELISA, immunofluorescence or plaque reduction neutralization tests (PRNT) (Mota et al., 2010; Silva-Fernandes et al., 2009; Abrahão et al., 2009a). Viruses can be isolated from vesicular secretions and scabs through the inoculation of permissive cell lines or the chorioallantoic membrane of embryonated hen's eggs (Leite et al., 2005; Abrahão et al., 2009a). Molecular and immunological techniques, including nested and semi-nested PCR (Abrahão et al., 2009a,d) and ELISA (Silva-Fernandes et al., 2009), have been widely applied in VACV diagnosis and research. The use of real-time PCR for the detection of VACV directly from lesions, without DNA or virus manipulation, was described by de Souza Trindade et al. (2008). In this work, de Souza Trindade and colleagues described a set of primers that allow for the identification of Br-VACV based on a deletion in the *A56R* gene. Similar approaches also use genetic markers to identify Br-VACV (Damaso et al., 2007).

Another technique used to identify OPV is microscopy. Viral particles obtained from the lesions may be visualized by electron microscopy after specimen fixation, staining and labeling (reviewed by Trindade et al., 2007b). A more elaborated alternative for viral visualization is atomic force microscopy (Fig. 1A and B) (Trindade et al., 2007b). Although microscopy is a widely accepted technique for virus identification in outbreak situations, this approach is not suitable for routine diagnostics.

5. The diversity of the Br-VACV is shown by molecular and biological characterization

During the last decade, several VACVs were isolated and characterized in Brazil (Damaso et al., 2000; de Souza Trindade et al., 2003, 2007; Leite et al., 2005; Trindade et al., 2006, 2009; Megid et al., 2008; Abrahão et al., 2009a; Medaglia et al., 2009). When outbreak notifications first began, the diagnosis of the etiologic agent was the priority; therefore, it was difficult to propose a hypothesis about the origin and genetic heterogeneity of the samples. Today, however, reasonable amounts of genetic and biological data allow a wider view of the viral polymorphisms and the relationships among these viruses.

In this context, the most important finding about the diversity of Br-VACV is that there is a clear phenotypic and genetic dichotomy among the viruses (Trindade et al., 2007a; Drumond et al., 2008; Ferreira et al., 2008a; Campos et al., 2011). This hypothesis has been supported by studies of the partial genomic sequencing of these viruses and by *in vivo* virulence studies (Trindade et al., 2007a; Drumond et al., 2008; Ferreira et al., 2008a). In recent years, several genes, including those for viral growth factor (VGF), thymidine kinase (TK), interferon resistance (E3L), EEV surface glycoprotein (B5R) and type I interferon-binding protein (B18R), have been used to characterize viral isolates (Damaso et al., 2000; de Souza Trindade et al., 2003, 2007; Leite et al., 2005; Trindade et al., 2006, 2009; Abrahão et al., 2009a; Medaglia et al., 2009; Campos et al., 2011).

Another genetic marker used in the characterization and differentiation of Br-VACV is the A-type inclusion body gene (ATI). This gene is truncated in all VACVs and is probably associated with the low conservation pressure on the open reading frame (Leite et al., 2007). Previous studies have indicated that there is genetic variability in the ATI gene among some Br-VACVs. To further investigate this variability, Leite et al. (2007) performed molecular analysis of the internal region of the ATI gene of eight Br-VACVs. Though the internal region of this gene was similar in SAV and the VACV Western Reserve (WR) strain, Muriae virus (MURV), GP2V, PSTV and ARAV all exhibited two blocks of deletions in the analyzed region (Leite et al., 2007). In addition, the ATI gene was almost entirely deleted from three other Br-VACV: VBH, GP1V and BAV (Leite et al., 2007). Therefore, ATI varies significantly among Br-VACVs, which reinforces the idea that most Br-VACVs are genetically heterogeneous and should be treated as separate entities rather than as isolates of the same strain in different areas and circumstances.

The most widely used genetic marker in Br-VACV studies is the gene encoding the viral hemagglutinin (*A56R*) (Fig. 3) (Damaso et al., 2000; de Souza Trindade et al., 2003; Leite et al., 2005; Trindade et al., 2006, 2009; Abrahão et al., 2009a; Medaglia et al., 2009). This gene deserves attention because many Br-VACV samples have a deletion of 6 amino acids (aa) at the C-terminus (Fig. 3) (Damaso et al., 2000; Abrahão et al., 2009a; Medaglia et al., 2009; Campos et al., 2011). The Br-VACV Araçatuba virus (ARAV), Cantagalo virus (CTGV), Passatempo virus (PSTV), GuaraniP2 virus (GP2V), Serro virus (S2V), Mariana virus (MARV) and Pelotas2 virus (P2V) (group 1, Fig. 4), among others, also have a deletion of 6 aa at position 251 in *A56R* (Damaso et al., 2000; de Souza Trindade et al., 2003; Leite et al., 2005; Trindade et al., 2006, 2009; Abrahão et al., 2009a; Campos et al., 2011). Despite this common deletion, the viruses have several unique nucleotide (nt) substitutions throughout the *A56R* gene. Hypothetically, these substitutions should allow some viruses to be identified as single

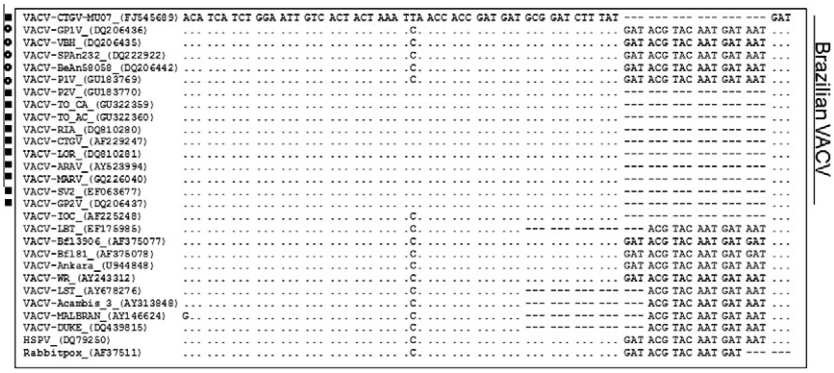

Fig. 3. A fragment of *A56R* nucleotide sequences of VACV samples and a comparison with the homologous gene sequences from several *orthopoxviruses*. The alignment shows the deletion signature region in several Br-VACV isolated during bovine vaccinia outbreaks. The alignments were made with ClustalW (Thompson et al., 1994) and MEGA version 3.1 software (Kumar et al., 2004).

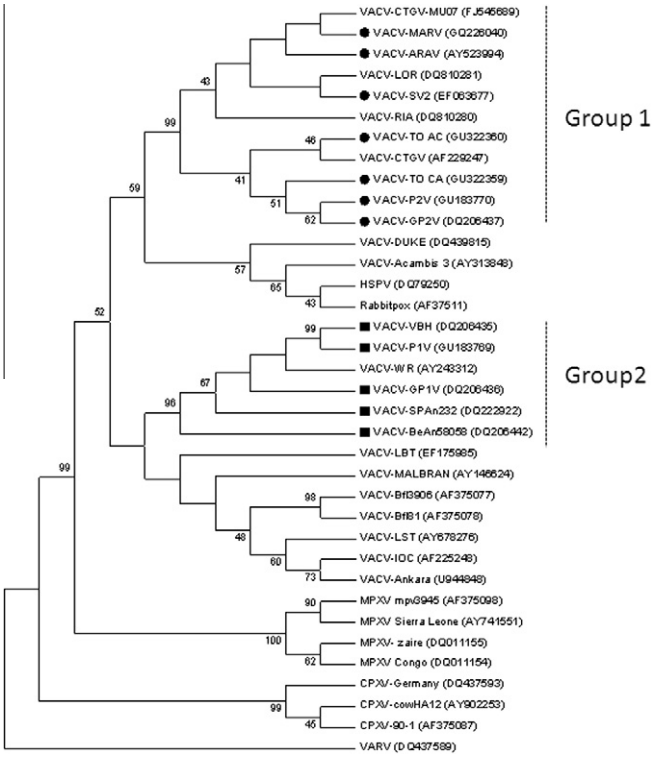

Fig. 4. The consensus bootstrap phylogenetic tree based on the nucleotide sequences of the *A56R* hemagglutinin (HA) gene. The tree was constructed with HA sequences by using the neighbor-joining method with 1000 bootstrap replicates and the Tamura 3-parameter model in MEGA version 3.1 software (Kumar et al., 2004). The bootstrap values >40% are shown. The nucleotide sequences were obtained from GenBank. The black dots and black squares indicate the Vaccinia virus (VACV) characterized by our lab. The Figure highlights the dichotomy among Br-VACV.

entities and to not be considered as multiple isolates of the same virus. Furthermore, some Br-VACVs do not exhibit a deletion in the *A56R* gene (group 2, Fig. 4); such Br-VACVs include GuaraniP1 virus (GP1), Belo Horizonte virus (VBH), Pelotas1 virus (P1V), and the viruses isolated during the 60's and 70's in the Brazilian forests: BeAn58058 virus (BAV) and SPAn-232 virus (SAV) (Trindade et al., 2004, 2006; Fonseca et al., 1998; da Fonseca et al., 2002). Phylogenetic analysis, based on the *A56R* gene sequences, shows a clear segregation of Br-VACV into two well defined groups according to the described gene deletion pattern (Fig. 4) (Trindade et al., 2007a; Drumond et al., 2008). Also, in the context of the *A56R* gene, some studies have examined the similarity between the Br-VACV and the viruses used as vaccines during the WHO smallpox eradication campaign. Drumond and colleagues analyzed *A56R* and several other genes present in the viral ITRs and demonstrated that Lister–Butantan (LST–BTT), a vaccine strain used in Brazil during the WHO campaign, did not cluster directly with the Br-VACV (Drumond et al., 2008). Although the IOC vaccine strain presents the same 18 nt deletion (Fig. 3), this strain presents nucleotide substitutions throughout the gene that are not shared by Br-VACV, which causes IOC to cluster with other vaccine strains (Trindade et al., 2007a; Drumond et al., 2008). Therefore, the most recent and comprehensive genetic studies suggest that the origin and natural history of the Br-VACV are distinct from those of the vaccine strains used in Brazil (Trindade et al., 2007a; Drumond et al., 2008).

The dichotomy of the Br-VACV, indicated mainly by the genetic analysis of the *A56R* gene, can also be extended to the biological level. Ferreira and colleagues (2008a) demonstrated that BALB/c mice that were intranasally infected with the Br-VACV exhibited one of two well defined outcomes: (a) acute respiratory illness followed by death or (b) complete absence of clinical symptoms. Interestingly, the clusters of viruses involved in each of these outcomes are exactly the same as those observed in the *A56R* phylogenetic analysis. Ferreira et al. (2008a) showed that GP1V, VBH, BAV and SAV cause clinical symptoms in mice, including an arched back, ruffled fur, laborious breathing, and decreased activities followed by death. Some of the infected mice exhibited outcomes such as balanoposthitis and periocular alopecia. Animals intranasally inoculated with ARAV, GP2V and PSTV did not show any apparent clinical signs (Ferreira et al., 2008a). Despite this remarkable difference in virulence and pathogenesis between the two groups, no difference in the severity of injuries in humans and cattle was observed or reported from the outbreaks during which the specimens were collected. A comprehensive list of some Br-VACVs isolated so far, together with the associated polymorphisms in ATI and HA genes, in addition to their virulence in mice are shown in Table 1.

Another special feature regarding the Br-VACV is the co-circulation and co-infection of distinct viral strains. The first reported case of this phenomenon was the isolation of two different VACVs (GuaraniP1 and GuaraniP2) from two neighboring properties, 10 km (6.25 miles) apart from each other, that were affected by BV during the same outbreak in Guarani county in 2001 (Trindade et al., 2006). In 2008, the VACV Pelotas1 virus and Pelotas2 virus were isolated from the same equine exanthematic lesion (Brum et al., 2010; Campos et al., 2011). Pelotas viruses presented 95.6% similarity in the *A56R* nt sequences (Campos et al., 2011), and Guarani viruses presented 95.1% similarity (Trindade et al., 2006).

Table 1
Isolated Br-VACV and the associated polymorphisms in the ATI (A26L) and HA (*A56R*) genes.

Isolate	Year/site of isolation	Associated host	ATI gene (nt)/protein	18 nt deletion in *A56R* (HA)	Virulence in BALB/c mice	Reference
BeAn58058 virus (BAV)	1963/Belém-PA	*Oryzomys* spp. (rodent)	Almost entirely deleted (~300 bp)	No	High	Fonseca et al. (1998)
SPAn-232 (SAV)	1960's/Cotia-SP	*Mus musculus* (Sentinel mice)	Similar to VACV-WR (~1.600 bp)	No	High	da Fonseca et al. (2002)
Belo Horizonte virus (VBH)	1993/Belo Horizonte-MG	*Mus musculus* (Laboratory Swiss mice)	Almost entirely deleted (~300 bp)	No	High	Diniz et al. (2001), Trindade et al. (2004)
Cantagalo virus (CTGV)[a]	1999/Cantagalo-RJ	*Bos taurus*	94 kDa protein	Yes	Unknown	Damaso et al. (2000)
Araçatuba virus (ARAV)[a]	1999/Araçatuba-SP	*Bos taurus*	Two blocks of deletions (~1.500 bp)	Yes	Low	de Souza Trindade et al. (2003)
Muriaé virus (MURV)[a]	2000/Muriaé-MG	*Homo sapiens*	Unknown	Yes	Unknown	Trindade et al. (2007a)
GuaraniP1 virus (GP1V)[a]	2001/Guarani-MG	*Bos taurus*	Almost entirely deleted (~300 bp)	No	High	Trindade et al. (2006)
GuaraniP2 virus (GP2V)[a]	2001/Guarani-MG	*Bos taurus*	Two blocks of deletions (~1.500 bp)	Yes	Low	Trindade et al. (2006)
Passatempo virus (PSTV)[a]	2003/ Passatempo-MG	*Bos taurus*	Two blocks of deletions (~1.500 bp)	Yes	Low	Leite et al. (2005)
Pelotas 1 virus (P1V)[b]	2008/Pelotas-RS	*Eqqus caballus*	Unknown	No	High	Brum et al. (2010), Campos et al. (2011)
Pelotas 2 (PV2)[b]	2008/Pelotas-RS	*Eqqus caballus*	Unknown	Yes	Low	Brum et al. (2010), Campos et al. (2011)
Mariana virus (MARV)	2008/Mariana-MG	*Mus musculus*, *Bos taurus*, *Homo sapiens*	Unknown	Yes	Low	Abrahão et al. (2009a)

[a] Isolated during bovine vaccinia outbreaks.
[b] Isolated from an exanthematic lesion in a Creole horse.

VACV co-infection with Pseudocowpox virus in humans and cattle was also described during a BV outbreak (Abrahão et al., 2010b). These findings indicate that intra- or inter-specific co-circulation/co-infections of poxviruses is relatively frequent in Brazilian rural areas. VACV and other poxviruses have a large range of hosts and could, theoretically, infect the same host. The consequences of co-infection are still unpredictable. However, the presence of several VACV subpopulations or of VACV and other virus species in the same lesion could lead to phenotype complementation and the replication of many viral variants, even those not adapted to a specific host.

Viral recombination cannot be neglected (McLysaght et al., 2003), as was suggested by Drumond and colleagues (2008). All of these factors could help maintain genetic diversity in poxvirus subpopulations or in species that randomly coexist and evolve together.

6. The epidemiological and ecological evidence of the Br-VACV spread

In the 1960s, a government-supported program was developed with the aim to search for and identify circulating arboviruses in specific parts of Brazil. The first Br-VACVs isolated in Brazil were found in this context. The BeAn58058 virus (BAV) was isolated from a wild rodent of the *Oryzomys* genus that was captured near the edge of a deforested area bordered by the rain forests in Para State, a part of the Brazilian Amazon basin. Additionally, the SPAn-232 (SAV) and Cotia viruses were repeatedly isolated from sentinel mice that were exposed at forested areas around the city of São Paulo in the southeastern region of Brazil (Lopes et al., 1965; Fonseca et al., 1998; da Fonseca et al., 2002).

During the modern history of smallpox vaccination, several strains of VACV showing different degrees of virulence were used around the world until the Global Program for Smallpox Eradication was created (Henderson, 2009). Under the rules of this globalized program, less virulent strains quickly became the official WHO choice for mass vaccination. Several of these strains were used in Brazil. During the 1970s and 1980s, some sporadic VACV infections affecting cows and humans were reported in several states around the country (Moussatché et al., 2008; Trindade et al., 2007a). At the beginning of the 1990s, a VACV was isolated from laboratory mice during a mousepox-like outbreak at the animal facility of the Biological Institute of the Federal University of Minas Gerais State (Diniz et al., 2001; Trindade et al., 2004).

After the WHO declared that smallpox had been eradicated in 1979, mass vaccination was stopped, and VACV manipulation was limited to laboratory environments, mostly as a tool for foreign protein expression. As mass vaccination was stopped, no sporadic outbreaks in bovine herds or humans were described until the end of the 1990s, when an increasing number of zoonotic VACV outbreaks affecting dairy cattle and their handlers were reported in different areas of the Brazilian countryside (Damaso et al., 2000; Schatzmayr et al., 2000; de Souza Trindade et al., 2003, 2007; Nagasse-Sugahara et al., 2004; Leite et al., 2005; Simonetti et al., 2007; Silva et al., 2008; Megid et al., 2008; Medaglia et al., 2009; Silva-Fernandes et al., 2009; Schatzmayr et al., 2009).

The Southeast region of Brazil, which contains many dairy properties, has been especially affected by the BV outbreaks (Fig. 5). The first BV cases were described in the states of Rio de Janeiro and São Paulo. However, within the past 11 years, several cases of bovine and human infections associated with similar outbreaks have been reported in other areas of the country. These outbreaks seem to be spreading radially from an epicenter in the southeast and central regions of Brazil. Recently, an outbreak affecting Creole horses was described in southern Brazil (Brum et al., 2010; Campos et al., 2011). VACV has also been recently detected in wild monkeys rescued from a flooded area in the Amazon region (Abrahão et al., 2010a). To date, BV is a multi-state disseminated disease that has officially been reported in 11 of the 26 Brazilian states. VACV is widely distributed in all geographical regions of Brazil, including at least five of the six national biomes (Fig. 5A), with emphasis on areas of intense anthropogenic disturbances, which are mainly represented by the areas of the tropical rainforest and the Brazilian

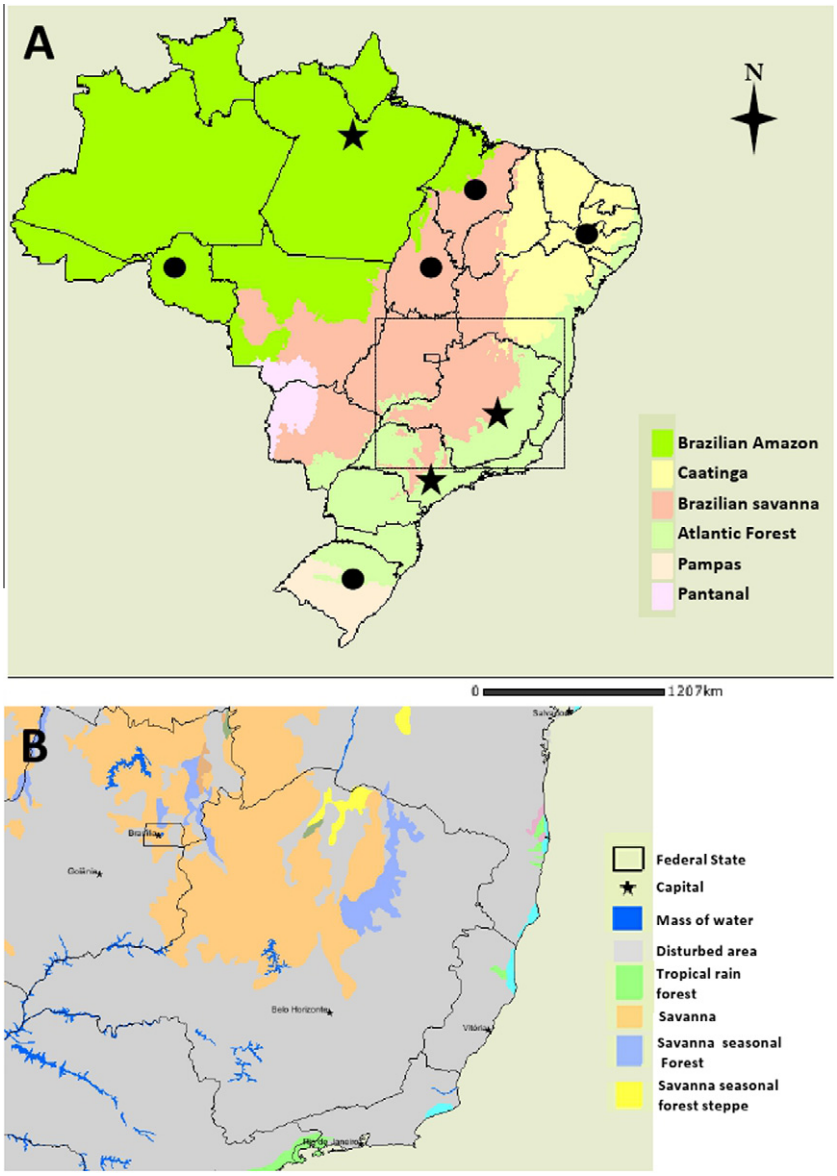

Fig. 5. (A) A map of Brazil showing the distribution of the six ecological biomes. The dotted box includes part of the central and southeast where the first outbreaks were reported in Brazil, in São Paulo, Rio de Janeiro, Minas Gerais, Goiás and Espírito Santo states. The black circles represent the states where outbreaks of bovine vaccinia have been reported in humans, cattle and horses. The stars represent the areas where the VACV has been isolated from rodents. (B) The dotted box shows the vegetation map of Brazil, highlighting the southeast where most outbreaks have been described. Note the large area of disturbed region (in gray). The maps have been modified from the Brazilian Institute for Geography and Statistics (IBGE), 2011 (http://www.ibge.gov.br).

savanna, and the transition regions between those biomes (da Fonseca et al., 2011) (Fig. 5B). In addition, the detection of VACV infections in hosts other than cattle and humans highlights the fact that the virus may jump between several mammalian host species across the country. Reinforcing these observations, a recent study demonstrated the presence of VACV in a peridomestic rodent (*Mus musculus*) that was trapped in a BV outbreak area (Abrahão et al., 2009a).

In Brazil, there is little information about the natural host of VACV. In addition to their presence in rodents, humans and barnyard animals such as cows and horses, VACVs have been detected in wild capuchin and black-howling monkeys in the Brazilian Amazon in northeastern Brazil (Abrahão et al., 2010a). The spread of VACV in northeastern Brazil has been reported, and cases of BV outbreaks in the Amazon region have also been described recently (Medaglia et al., 2009; Mota et al., 2010; Quixabeira-Santos et al., 2011). The relationship between infected monkeys and the emergence of VACV in the rural regions of Brazil is still unknown, but these findings clearly indicate that the virus is well established in the wild and in rural environments. The anthropogenic disturbance of the Amazon ecosystem and the expansion of the agricultural and livestock areas increase contact between the wildlife and rural populations (Abrahão et al., 2010a; Mota et al., 2010); we believe that this may underlie the emergence of BV in the Brazilian Amazon basin. There is a consensus that the emergence of infectious diseases is directly related to social, economic and environmental determinants (Jones et al., 2008). In several cases, the advancement of agriculture and livestock in natural areas has generated contact between humans and their domestic animals with wild animal populations in their habitats. This close contact facilitates the spread of infectious and parasitic agents to new hosts and environments, thus establishing new relationships between hosts and parasites and new ecological niches in the chain of disease transmission. As a consequence of these interactions, epidemic zoonoses such as BV emerge.

As previously discussed, VACV has been isolated from several species of rodents, including lab mice, sentinel mice, peridomestic and wild mice. Although several VACVs have been isolated from lab, sentinel and peridomestic rodents (Trindade et al., 2004; da Fonseca et al., 2002; Abrahão et al., 2009a), BAV was isolated from a wild rodent of the *Oryzomys* genus (Fonseca et al., 1998). This genus comprises several wild species that circulate in a large region of the country and live in varied habitats, ranging from tropical rainforest to savanna landscapes such as the Brazilian Cerrado and Caatinga, including border regions between different phyto-ecological systems (Fundação Nacional de Saúde, 2002). These rodents may have access to peridomestic areas through the expansion of farming areas on the forest edge (Abrahão et al., 2009a). Zoonotic viral agents associated with rodents have considerable relevance to public health in the tropical regions of the world (Emonet et al., 2009; Dearing and Dizney, 2010; Charrel and de Lamballerie, 2010). Because the mammalian fauna of Brazil is extremely rich, comprising approximately 12% of the 5416 mammalian species described worldwide (Wilson and Reeder, 2005), other species of wild and synanthropic rodents should also be evaluated as potential natural VACV reservoirs.

7. Combining epidemiological and molecular data: where does Br-VACV come from?

One of the most intriguing questions about Br-VACV biology is, "where do these isolates come from?" Currently, there are two non-exclusive hypotheses to explain the origins of the Br-VACV. The first one is that Br-VACVs derived from a vaccine strain that escaped to the wild when smallpox vaccination was routine, accumulated mutations during several transmission cycles in wild host populations, and recently emerged, causing the BV outbreaks (Damaso et al., 2000; Simonetti et al., 2007; Schatzmayr et al., 2009; Megid et al., 2008; Lobato et al., 2005; Leite et al., 2005; de Souza Trindade et al., 2003). According to this hypothesis, there is little genetic and phenotypic variation between the virus isolated from BV outbreaks and the vaccine strains used in Brazil during the WHO smallpox vaccination campaign. The second hypothesis is that there is an existing population with genetic and phenotypic diversity of the VACV strains that circulates in the unknown natural reservoir populations. Some of these strains are then transmitted to the accidental hosts (cows and humans) depending on biological and geographical conditions.

The best way to answer this question would be to put together data collected from the epidemiological and molecular fields. Using this approach, we believe that the second hypothesis would better explain the epidemiology of BV. First, there is extensive genetic and phenotypic diversity among Br-VACVs, and the isolates are phylogenetic separated from vaccine strains used in Brazil (Fig. 4) (Trindade et al., 2007a; Drumond et al., 2008; Ferreira et al., 2008a). Moreover, VACV isolated from wild animals also are quite different compared with the vaccine strains (Abrahão et al., 2010a). Additionally, it is well known that Br-VACVs cause an acute disease, which does not have a long chain of transmission between cows and humans. This means that these pathogens have a basic reproductive number (R_0) below one (Damaso et al., 2000; Schatzmayr et al., 2000; de Souza Trindade et al., 2003, 2007; Nagasse-Sugahara et al., 2004; Leite et al., 2005; Simonetti et al., 2007; Silva et al., 2008; Megid et al., 2008; Medaglia et al., 2009; Silva-Fernandes et al., 2009; Schatzmayr et al., 2009). In theory, each one of the isolates from the BV outbreaks is the result of a new introduction of the virus from the wild reservoir to the bovine and human populations, which is probably related to the intensification of anthropogenic activities in Brazil (Abrahão et al., 2009a). The finding of two different VACV strains isolated from two close rural proprieties at the same time seems to corroborate this (Trindade et al., 2006). If this hypothesis is true, it would be quite unlikely that some VACV strains isolated in recent years may represent repeated isolation of the same vaccine strain.

8. A hypothesis about the VACV transmission cycle

Despite the recent advances in the molecular characterization of the Br-VACV, some important biological aspects remain unknown. Due to the large VACV host range and a lack of knowledge regarding all of the possible routes of VACV transmission, it is difficult to propose a solid and complete hypothesis of the VACV cycle of life. In most cases, the spread of BV during an outbreak is related to an infected milker with hand lesions that spread the virus to the herd during the milking process (Lobato et al., 2005; Silva-Fernandes et al., 2009). Most of the epidemiological retrospective studies point to infected milkers and cattle commercialization as the initial causes of BV outbreaks; however, the origins of the outbreaks are unknown in some cases (Trindade et al., 2006; Leite et al., 2005; Lobato et al., 2005; Silva-Fernandes et al., 2009). An infected milker sheds virus particles during some weeks, which leads to the infection of a high percent of the herd by occupational contact (Lobato et al., 2005). The "milker" factor can worsen the BV problem if the same person keeps contact with the animals from distinct farms, which is very common in Brazilian rural areas. Therefore, direct contact between cattle and humans is the most described way of Br-VACV transmission. Cattle-to-cattle transmission is frequently reported, especially during calf feeding (Leite et al., 2005); human-to-human transmission was also described (Batista et al., 2009).

However, some VACV outbreaks are temporally and spatially distant from the previously reported BV outbreak areas. In those cases, the focal origin of the outbreaks is sometimes unknown. Moreover, BV usually occurs during the dry season when some Brazilian biomes have a scarcity of food, which leads some wild species to search for food in farm storehouses and corrals. Rats, mice, opossums, foxes, wild dogs and small cats are frequently observed around farming areas (Abrahão et al., 2009a). In theory, some of these species, especially rodents, could be VACV reservoirs. A recent study developed by our group showed that peridomestic rodents, such as the *Mus musculus* and the *Rattus rattus* species, could be carriers of VACV between forests and farm facilities. In this study, a VACV named Mariana virus was concomitantly isolated from a peridomestic mouse, a milker and an infected cow, indicating that rodents around the farms could represent a hypothetical risk factor (Abrahão et al., 2009a). Moreover, the detection of VACV in rodents and monkeys in the Brazilian forests indicate that VACV may have circulation in the wild (Lopes et al., 1965; Abrahão et al., 2010a). The impact of anthropogenic activities on the VACV emergence from the wild is a matter of investigation.

By taking all of this information into account, a hypothetical VACV (Fig. 6) transmission model in which peridomestic rodents act as a connection between wildlife and animals in the rural environment has been suggested (Abrahão et al., 2009a). According to this ecological model of VACV transmission, peridomestic rodents could be infected by wild animals around the farms through territorial fights, aspiration or ingestion of contaminated excrement, consumption of contaminated carcasses or consumption of food containing the saliva of an infected animal. Some infected peridomestic rodents eventually return to the farms, introducing VACV into the bovine and human populations by excrement or direct contact. Peridomestic mice and rats could be infected with VACV after coming into contact with bovine or human scab fragments or contaminated milk, excrement or fomites. This hypothesis can

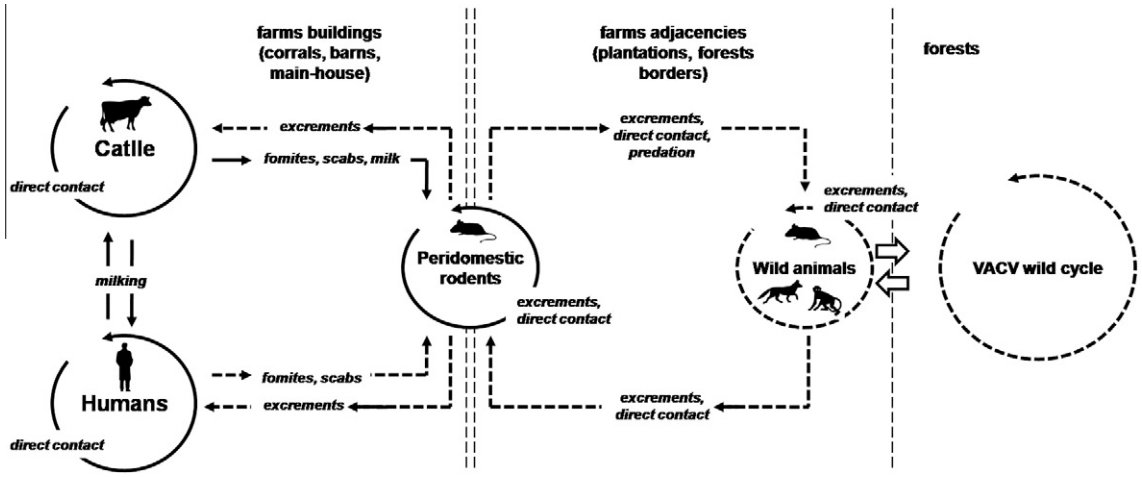

Fig. 6. A hypothetical model of the VACV transmission cycle. Peridomestic rodents can promote the transmission of VACV between wild animals and cattle or humans because they have access to farm facilities and plantations. Solid lines indicate experimentally determined data, and dashed lines represent hypothetical propositions that are still under investigation.

be partially supported by a recent study in which D'Anunciação and colleagues (2011) showed the infection of BALB/c mice exposed to the feces of cows that had been experimentally infected with VACV-GP2V (D'Anunciação et al., in press). Subsequently, the infected peridomestic rodents could spread VACV to wild animals when they are predated, via their feces or carcasses, or during fights. Previous studies demonstrated that BALB/c mice intranasally inoculated with Br-VACV shed viral particles and DNA in their feces for at least 30 days post-infection (d.p.i.). Sentinel mice exposed to murine excrement contaminated with VACV also become infected and shed viral particles and DNA in their feces (Ferreira et al., 2008b). A similar viral shedding profile was shown for other OPVs in rats (Maiboroda, 1982). Moreover, the long-lasting stability of VACV in murine feces exposed to environmental conditions was recently demonstrated, which raised important questions related to the viral circulation and maintenance among peridomestic and wild rodents (Abrahão et al., 2009b; Ferreira et al., 2008b). Although less probable, the possibility of direct VACV transmission between wild animals and humans/cattle cannot be ruled out (Abrahão et al., 2009a). Additional studies are required to elucidate the complex VACV transmission dynamics, especially when considering the virus's large host range.

9. How is the disease controlled in the host?

The progression of the disease and the clinical signs observed in these Br-VACV outbreaks are invariably similar to the clinical descriptions of CPXV infections in Europe (Honlinger et al., 2005; Coras et al., 2005).

Neutralizing antibodies were detected (Schatzmayr et al., 2009; Silva-Fernandes et al., 2009; de Souza Trindade et al., 2007; Abrahão et al., 2009a), and IgG or IgM have been detected in patients by ELISA (Silva-Fernandes et al., 2009). DNAemia has also been described in the sera of naturally infected humans (Silva-Fernandes et al., 2009; Abrahão et al., 2010b), intranasally infected BALB/c mice (Ferreira et al., 2008a) and wild monkeys (Abrahão et al., 2010a), as has been described for the CPXV (Nitsche et al., 2007).

The immune mechanisms of protection in secondary poxvirus infections are well known, as antibodies are thought to be at the core of long-term protection against subsequent OPV infections in previously immunized individuals (Hammarlund et al., 2003; Belyakov et al., 2003; Edghill-Smith et al., 2005; Panchanathan et al., 2010). However, the mechanism of virus clearing in primary poxviruses' infections is not known. In this respect, essential and complementary functions for both the innate and adaptive responses have been suggested (Lauterbach et al., 2010; Mota et al., 2011), but the identification of the isolated functions for each compartment of the immune response is controversial. In VACV infections of mice, the T cell response seems to be sufficient for virus clearing and host survival in models of infection that mimic the scarification route used to vaccinate humans against smallpox (Neyts et al., 2004; Mota et al., 2011). Using other routes of infection, Xu and coworkers showed that abrogation of T $CD4^+$ and B cell responses led to a more severe infection outcome (Xu et al., 2004). However, when species-specific OPV, such as ECTV, are used to infect mice, both CD8+ cell function and antibody responses are essential, as abrogation of these functions renders animals unable to contain and clear the infection (Fang and Sigal, 2005; Chaudhri et al., 2006).

The Br-VACV outbreaks represent an important opportunity to understand aspects of the human immune responses during an acute OPV' infection on a population scale. We have recently analyzed aspects of the immune responses to VACV during zoonotic BV outbreaks in the rural regions of southeast Brazil. Peripheral blood mononuclear cells (PBMCs) from an infected patient showed low proliferative responses and low production of cytokines after stimulation with VACV antigens *in vitro*. The levels of IFN-γ production and the levels of other T cell activation markers, such as CD25, were surprisingly lower in the infected patient than in the uninfected control subjects (Trindade et al., 2009). Additionally, analysis of cell populations and subpopulations of the PBMCs of a large group of infected individuals presented a marked decrease in the activation status of B cells, T CD4+ cells and CD14+ cells when compared to non-infected controls. Our group has recently submitted these results for publication (Gomes et al., submitted).

10. Therapeutic agents to treat OPV infections

The threat of an intentional release of VARV and the recent emergence of zoonotic MPXV in Africa (Di Giulio and Eckburg, 2004) and VACV in Asia and Brazil (Bhanuprakash et al., 2010; Trindade et al., 2007a) has further increased the public health relevance of OPV. Moreover, patients with an immune deficiency are more likely to have severe outcomes when infected with these zoonotic viruses or when vaccinated against smallpox (Bray and Wright, 2003). Hence, there is a need for secure and effective drugs to treat acute OPV infections (Parker et al., 2008). Drugs with proved antipoxviral activity *in vitro* and their modes of action are

Table 2
Anti-poxviral compounds classified by their mode of action.

Therapeutic agent	Mode of action	Effective concentration *in vitro*	Clinical trial	Adverse reactions	References
Cell penetrating peptides (CPP)	Inhibitor of viral entry	15 μM	No	Not tested *in vivo*	Altmann et al. (2009)
Nigericin	Inhibitor of early gene expression and DNA replication	7.9 nM in human cells (HeLa)	No	Highly toxic *in vivo*	Myskiw et al. (2010)
Cidofovir (CDV), CMX001	Nucleotide analogs (DNA replication inhibitors)	CDV: 46 μM; CMX001: 0.8 μM	Yes. Currently licensed for the treatment of CMV retinitis (Vistidine™). CMX001 is regulated for smallpox under the "Animal Rule" efficacy	Resistant strains may emerge; Nephrotoxicity (can be alleviate with the use of probenecid). CMX001 is not toxic and is orally available	De Clercq et al. (1986), Lanier et al. (2010)
STI-571 (Gleevec™)	Inhibitors of virus release and spread	10 μM in simian cells (BSC-40)	No for poxvirus treatment. Licensed for the treatment of certain types of cancer.	Inhibition of the physiological functions played by EGFR and Abl family kinases in non-infected cells	Reeves et al. (2011)
Gefitinib (Iressa™)	Inhibitors of virus release and spread	10 μM	No for poxvirus treatment. Licensed in US and Europe for the treatment of patients with non-small cell lung cancer (NSCLC)	Inhibition of the physiological functions played by EGFR and Abl family kinases in non-infected cells	Langhammer et al. (2011)
ST-246	Inhibitors of virus release and spread (acts on F13L ORF of VACV)	0.01 μM in VERO	FDA phase II clinical trials	Resistant strains may emerge	Yang et al. (2005)
RNA interference (RNAi)	Inhibition of protein expression	As low as 1 nm	No	Not described	Dave et al. (2006), Vigne et al. (2008, 2009)
Imiquimod	Immune Response Modifiers (IRMs)	1–5% topical cream	Licensed for the treatment of genital warts caused by HPV. A few studies for the treatment of poxvirus infections	Moderate/severe local inflammatory response	Tarbet et al. (2011)
Ethnopharmacological compounds	Unknown	Vary. Between 20 and 50 μg/ml in most studies	No	Not tested *in vivo*	Valadares et al. (2009), Brandao et al. (2010a,b)

described in Table 2 and Fig. 2. In the main text, only those drugs that are the most promising for use in humans are discussed further. In the context of the increasing number of BV outbreaks, the identification of possible therapeutic agents is important.

10.1. Cidofovir (CDV)

Cidofovir (CDV) is an acyclic nucleoside phosphonate that has a broad activity against several DNA viruses (De Clercq et al., 1986) and acts as an inhibitor of viral DNA polymerases (De Clercq, 2002). CDV is the only drug now licensed to treat OPV infection under the Investigational New Drug status (Parker et al., 2008). CDV is efficient both *in vitro* (Keith et al., 2003; Jesus et al., 2009) and *in vivo* as a prophylactic and therapeutic drug in mice infected with VACV and CPXV (Quenelle et al., 2003, 2004; Smee et al., 2004; Neyts et al., 2004). Furthermore, CDV has proven efficacy against MPXV infection in cynomolgus monkeys (Stittelaar et al., 2006). Topical CDV has been used in patients with recalcitrant moluscum contagiosum (Toutous-Trellu et al., 2004) and orf virus infections (Geerinck et al., 2001). However, CDV has poor oral availability and some degree of nephrotoxicity, which can be alleviated by the concomitant use of probenecid. Another drawback is the generation of viral resistance to the drug, which maps to two aa substitutions in the viral DNA polymerase (Gammon et al., 2008). However, all mutant viruses resistant to CDV are less virulent than wild-type viruses in mice (Gammon et al., 2008).

10.2. Cmx001

Some chemically modified CDVs have been described, such as the derivate CMX001 (Lanier et al., 2010; Sliva and Schnierle, 2007; Keith et al., 2003), which can be administered orally and is less nephrotoxic and has a much lower EC_{50} than CDV (Table 2) (Lanier et al., 2010).

10.3. Inhibitors of Src and Abl family kinases

OPVs have a complex morphogenetic pathway that includes the polymerization of actin tails, which propel virus progeny into neighboring cells (Fig. 2). The nucleation of actin tails requires the phosphorylation of the A36 protein by Src and Abl family kinases. Inhibitors of Abl family kinases, such as STI-571 (Gleevec), have been shown to block EEV release and limit the spread of infection *in vivo* (Reeves et al., 2005). It has been reported that VARV and MPXV use this conserved pathway to release CEVs and that an inhibitor of both Src and Abl family kinases (Dasatinib, also called Sprycel) limits the spread of VACV, VARV and MPXV *in vitro* (Reeves et al., 2011).

10.4. Gefitinib

VACVs and other OPVs have evolved several mechanisms to induce a proliferative state in infected and neighboring cells, including the production of the vaccinia growth factor (VGF), an early viral protein homolog to the epidermal growth factor (EGF) of mammals (de Magalhaes et al., 2001; Andrade et al., 2004). VGF is involved in pathogenesis presumably by activating the proliferative and anti-apoptotic state in the cell after binding to the EGF receptor (Tzahar et al., 1998; Lai and Pogo, 1989). Gefitinib (Iressa™) is an orally available small molecule tyrosine kinase inhibitor that is licensed in the US and Europe for the treatment of patients with non-small cell lung cancer. Treatment of human HepG2 cells with gefitinib at concentrations as low as 10 μM decreases the replication and plaque size of VACV and CPXV (Langhammer et al., 2011).

10.5. St-246

ST-246 is a small molecular compound that was identified in a high-throughput screen for inhibitors of the cytopathic effects of OPV (Yang et al., 2005). This compound is efficient *in vitro* against several OPVs (Duraffour et al., 2007; Smith et al., 2009). ST-246 prevents the morbidity and mortality of VACV infection in mice (Berhanu et al., 2009, 2010; Grosenbach et al., 2010), MPXV infection in ground squirrels (Sbrana et al., 2007), and VARV or MPXV infections in non-human primates (Huggins et al., 2009; Jordan et al., 2009). Although only a few human cases of adverse events after smallpox vaccination were successfully treated with ST-246 (Bristol, 2007; Vora et al., 2008), phase II clinical trials of ST-246 as an antipoxviral drug are now being conducted by the Food and Drug Administration (FDA). VACV strains resistant to ST-246 have also been described (Yang et al., 2005) and have a single aa change within the protein encoded by the F13L ORF in VACV, which is necessary for viral extracellular release and cell-to-cell spreading (Blasco and Moss, 1991). This issue can be circumvented by concomitant use with other OPV drugs (Quenelle et al., 2007).

10.6. Imiquimod

Imiquimod (Aldara™) is a small molecular compound that belongs to the family of imidazoquinolines that induce cytokine production upon binding to the Toll-like receptor 7 (TLR7) and signaling through the adaptor protein MyD88 (Hemmi et al., 2002). The induction of type I interferon (IFNαβ), interleukin (IL) 6, IL-12 and tumor necrosis factor alpha (TNFα) by imiquimod has been observed both in humans and in animal studies (Testerman et al., 1995; Reiter et al., 1994; Wagner et al., 1999). Recently, 1% imiquimod cream was successfully applied for the topical treatment of VACV cutaneous infection in chemically immunosuppressed animals. However, increasing the concentration of imiquimod from 1% to 5% led to a decrease in its antiviral activity (Tarbet et al., 2011). Imiquimod has been demonstrated to be beneficial in the treatment of infections caused by viruses belonging to other genera of the poxvirus family, specifically *Molluscum contagiosum* (MC) (Lin et al., 2010; Arican, 2006; Skinner, 2002) and the Parapoxvirus species (Ara et al., 2008; Lederman et al., 2007; Erbagci et al., 2005; Frandsen et al., 2011). Double-blind, randomized studies showed that imiquimod is promising for the treatment of *Molluscum contagiosum* infections in humans (Syed et al., 1998; Theos et al., 2004).

10.7. Ethnopharmacology and anti-poxvirus compounds

The empirical evaluation of plant extracts based on traditional knowledge is among the most promising sources of new compounds with pharmacological potential, especially for the treatment of infectious diseases. Several studies by our group have focused on plant extracts and derivatives for the discovery of new compounds with antiviral activity against OPV. Antiviral activity against VACV-WR *in vitro* has been demonstrated for ethanolic extracts of *Solanum paniculatum* (EC_{50} of 177.0 ± 3.3 μg/ml) (Valadares et al., 2009), *Polygonum spectabile* (EC_{50} < 30 μg/ml) (Brandao et al., 2010a), *Zeyheria tuberculosa* (EC_{50} of 23.2 ± 2.5 μg/ml) and *Arrabidaea sceptrum* (EC_{50} 40.6 ± 2.1 μg/ml) (Brandao et al., 2010b).

11. Concluding remarks

The study of zoonotic VACVs in Brazil has led to several conclusions about the epidemiology of the related disease and the evolution of these important human pathogens: (i) these viruses present a marked genetic and phenotypic diversity that are emerging, (ii) they constitute a defined risk to people and livestock, (iii) the circulation of these viruses in wildlife and peridomestic species suggests that they will persist as emerging pathogens, and (iv) there are currently no good medical interventions for serious clinical events, although some compounds are undergoing clinical trials and may soon be licensed for use in the treatment of OPV infections.

References

Abrahão, J.S., Guedes, M.I., Trindade, G.S., Fonseca, F.G., Campos, R.K., Mota, B.F., Lobato, Z.I., Silva-Fernandes, A.T., Rodrigues, G.O., Lima, L.S., Ferreira, P.C., Bonjardim, C.A., Kroon, E.G., 2009a. One more piece in the VACV ecological puzzle: could peridomestic rodents be the link between wildlife and bovine vaccinia outbreaks in Brazil? PLoS One 19, e7428.

Abrahão, J.S., Trindade Gde, S., Ferreira, J.M., Campos, R.K., Bonjardim, C.A., Ferreira, P.C., Kroon, E.G., 2009b. Long-lasting stability of Vaccinia virus strains in murine feces: implications for virus circulation and environmental maintenance. Arch. Virol. 154, 1551–1553.

Abrahão, J.S., Oliveira, T.M., Campos, R.K., Madureira, M.C., Kroon, E.G., Lobato, Z.I., 2009c. Bovine vaccinia outbreaks: detection and isolation of vaccinia virus in milk samples. Foodborne Pathog. Dis. 6, 1141–1146.

Abrahão, J.S., Lima, L.S., Assis, F.L., Alves, P.A., Silva-Fernandes, A.T., Cota, M.M., Ferreira, V.M., Campos, R.K., Mazur, C., Lobato, Z.I., Trindade, G.S., Kroon, E.G., 2009d. Nested-multiplex PCR detection of Orthopoxvirus and Parapoxvirus directly from exanthematic clinical samples. Virol. J. 6, 140.

Abrahão, J.S., Silva-Fernandes, A.T., Lima, L.S., Campos, R.K., Guedes, M.I., Cota, M.M., Assis, F.L., Borges, I.A., Souza-Júnior, M.F., Lobato, Z.I., Bonjardim, C.A., Ferreira, P.C., Trindade, G.S., Kroon, E.G., 2010a. Vaccinia virus infection in monkeys, Brazilian Amazon. Emerg. Infect. Dis. 16, 976–979.

Abrahão, J.S., Silva-Fernandes, A.T., Assis, F.L., Guedes, M.I., Drumond, B.P., Leite, J.A., Coelho, L.F., Turrini, F., Fonseca, F.G., Lobato, Z.I., Madureira, M., Ferreira, P.C., Bonjardim, C.A., Trindade, G.S., Kroon, E.G., 2010b. Human Vaccinia virus and Pseudocowpox virus co-infection: clinical description and phylogenetic characterization. J. Clin. Virol. 48, 69–72.

Altmann, S.E., Jones, J.C., Schultz-Cherry, S., Brandt, C.R., 2009. Inhibition of Vaccinia virus entry by a broad spectrum antiviral peptide. Virology 388, 248–259.

Andrade, A.A., Silva, P.N., Pereira, A.C., De Sousa, L.P., Ferreira, P.C., Gazzinelli, R.T., Kroon, E.G., Ropert, C., Bonjardim, C.A., 2004. The vaccinia virus-stimulated mitogen-activated protein kinase (MAPK) pathway is required for virus multiplication. Biochem. J. 381, 437–446.

Ara, M., Zaballos, P., Sanchez, M., Querol, I., Zubiri, M.L., Simal, E., Hörndler, C., 2008. Giant and recurrent orf virus infection in a renal transplant recipient treated with imiquimod. J. Am. Acad. Dermatol. 58, S39–S40.

Arican, O., 2006. Topical treatment of molluscum contagiosum with imiquimod 5% cream in Turkish children. Pediatr. Int. 48, 403–405.

Batista, V.H., Scremin, J., Aguiar, L.M., Schatzmayr, H.G., 2009. Vulvar infection and possible human-to-human transmission of bovine poxvirus disease. Virus Rev. Res. 14, 1–10.

Belyakov, I.M., Earl, P., Dzutsev, A., Kuznetsov, V.A., Lemon, M., Wyatt, L.S., Snyder, J.T., Ahlers, J.D., Franchini, G., Moss, B., Berzofsky, J.A., 2003. Shared modes of protection against poxvirus infection by attenuated and conventional smallpox vaccine viruses. Proc. Natl. Acad. Sci. USA 100, 9458–9463.

Berhanu, A., King, D.S., Mosier, S., Jordan, R., Jones, K.F., Hruby, D.E., Grosenbach, D.W., 2009. ST-246 inhibits in vivo poxvirus dissemination, virus shedding, and systemic disease manifestation. Antimicrob. Agents Chemother. 53, 4999–5009.

Berhanu, A., King, D.S., Mosier, S., Jordan, R., Jones, K.F., Hruby, D.E., Grosenbach, D.W., 2010. Impact of ST-246(R) on ACAM2000 smallpox vaccine reactogenicity, immunogenicity, and protective efficacy in immunodeficient mice. Vaccine 29, 289–303.

Bhanuprakash, V., Venkatesan, G., Balamurugan, V., Hosamani, M., Yogisharadhya, R., Gandhale, P., Reddy, K.V., Damle, A.S., Kher, H.N., Chandel, B.S., Chauhan, H.C., Singh, R.K., 2010. Zoonotic infections of buffalopox in India. Zoonoses Public Health 57, e149–e155.

Blasco, R., Moss, B., 1991. Extracellular vaccinia virus formation and cell-to-cell virus transmission are prevented by deletion of the gene encoding the 37, 000-Dalton outer envelope protein. J. Virol. 65, 5910–5920.

Brandao, G.C., Kroon, E.G., Duarte, M.G., Braga, F.C., Filho, J.D., de Oliveira, A.B., 2010a. Antimicrobial, antiviral and cytotoxic activity of extracts and constituents from Polygonum spectabile Mart. Phytomedicine 17, 926–929.

Brandao, G.C., Kroon, E.G., dos Santos, J.R., Stehmann, J.R., Lombardi, J.A., Braga de Oliveira, A., 2010b. Antiviral activity of Bignoniaceae species occurring in the State of Minas Gerais (Brazil): part 1. Lett. Appl. Microbiol. 51, 469–476.

Bray, M., Wright, M.E., 2003. Progressive vaccinia. Clin. Infect. Dis. 36, 766–774.

Bristol, N., 2007. Adverse event related to smallpox vaccination. Disaster Med. Public Health Prep. 1, 11–12.

Brum, M.C., Anjos, B.L., Nogueira, C.E., Amaral, L.A., Weiblen, R., Flores, E.F., 2010. An outbreak of orthopoxvirus-associated disease in horses in southern Brazil. J. Vet. Diag. Invest. 22, 143–147.

Campos, R.K., Brum, M.C., Nogueira, C.E., Drumond, B.P., Alves, P.A., Siqueira-Lima, L., Assis, F.L., Trindade, G.S., Bonjardim, C.A., Ferreira, P.C., Weiblen, R., Flores, E.F., Kroon, E.G., Abrahão, J.S., 2011. Assessing the variability of Brazilian

Vaccinia virus isolates from a horse exanthematic lesion: coinfection with distinct viruses. Arch. Virol. 156, 275–283.
Charrel, R.N., de Lamballerie, X., 2010. Zoonotic aspects of arenavirus infections. Vet. Microbiol. 140, 213–220.
Chaudhri, G., Panchanathan, V., Bluethmann, H., Karupiah, G., 2006. Obligatory requirement for antibody in recovery from a primary poxvirus infection. J. Virol. 80, 6339–6344.
Condit, R.C., Moussatche, N., Traktman, P., 2006. In a nutshell: structure and assembly of the vaccinia virion. Adv. Virus Res. 66, 31–124.
Coras, B., Essbauer, S., Pfeffer, M., Meyer, H., Schröder, J., Stolz, W., Landthaler, M., Vogt, T., 2005. Cowpox and a cat. Lancet 365, 446.
Costa, R.V.C., Simonetti, B.R., Abreu, D.C., Simonetti, J.P., Gonçalves, M.C.R., Silva, M.E.V., Brust, L.A.C., Barth, O.M., Schatzmayr, H.G., 2007. Animal infections by vaccinia-like viruses in the state of Rio de Janeiro: 2 - Paraíba river valley. Virus Rev. Res. 12, 33–37.
Damaso, C.R., Esposito, J.J., Condit, R.C., Moussatché, N., 2000. An emergent poxvirus from humans and cattle in Rio de Janeiro State: Cantagalo virus may derive from Brazilian smallpox vaccine. Virology 277, 439–449.
Damaso, C.R., Reis, S.A., Jesus, D.M., Lima, P.S., Moussatché, N., 2007. A PCR-based assay for detection of emerging vaccinia-like viruses isolated in Brazil. Diagn. Microbiol. Infect. Dis. 57, 39–46.
da Fonseca, F.G., Trindade, G.S., Silva, R.L., Bonjardim, C.A., Ferreira, P.C., Kroon, E.G., 2002. Characterization of a vaccinia-like virus isolated in a Brazilian forest. J. Gen. Virol. 83, 223–228.
da Fonseca, F.G., Kroon, E.G., Nogueira, M.L., Trindade, G.S., 2011. Zoonotic vaccinia virus outbreaks in Brazil: a review. Future Virology 6, 697–707.
D'Anunciação L., Guedes M.I.M., Oliveira T.L., Rehfeld I., Bonjardim C.A., Ferreira P.P., Trindade G.S., Lobato Z.P., Kroon E.G., Abrahão J.S. Filling one more gap: experimental evidence of horizontal transmission of Vaccinia virus between bovines and rodents. Vector Borne Zoonotic Dis. (in press).
Dave, R.S., McGettigan, J.P., Qureshi, T., Schnell, M.J., Nunnari, G., Pomerantz, R.J., 2006. SiRNA targeting vaccinia virus double-stranded RNA binding protein [E3L] exerts potent antiviral effects. Virology 348, 489–497.
Dearing, M.D., Dizney, L., 2010. Ecology of hantavirus in a changing world. Ann. N. Y. Acad. Sci. 1195, 99–112.
De Clercq, E., Holy, A., Rosenberg, I., Sakuma, T., Balzarini, J., Maudgal, P.C., 1986. A novel selective broad-spectrum anti-DNA virus agent. Nature 323, 464–467.
De Clercq, E., 2002. Cidofovir in the treatment of poxvirus infections. Antiviral Res. 55, 1–13.
de Magalhaes, J.C., Andrade, A.A., Silva, P.N., Sousa, L.P., Ropert, C., Ferreira, P.C., Kroon, E.G., Gazzinelli, R.T., Bonjardim, C.A., 2001. A mitogenic signal triggered at an early stage of vaccinia virus infection: implication of MEK/ERK and protein kinase A in virus multiplication. J. Biol. Chem. 276, 38353–38360.
de Oliveira, T.M., Rehfeld, I.S., Siqueira, J.M., Abrahão, J.S., Campos, R.K., dos Santos, A.K., Cerqueira, M.M., Kroon, E.G., Lobato, Z.I., 2010. Vaccinia virus is not inactivated after thermal treatment and cheese production using experimentally contaminated milk. Foodborne Pathog. Dis. 7, 1491–1496.
de Souza Trindade, G.S., da Fonseca, F.G., Marques, J.T., Nogueira, M.L., Mendes, L.C., Borges, A.S., Peiró, J.R., Pituco, E.M., Bonjardim, C.A., Ferreira, P.C., Kroon, E.G., 2003. Araçatuba virus: a vaccinia like virus associated with infection in humans and cattle. Emerg. Infect. Dis. 9, 155–160.
de Souza Trindade, G., Drumond, B.P., Guedes, M.I., Leite, J.A., Mota, B.E., Campos, M.A., da Fonseca, F.G., et al., 2007. Zoonotic vaccinia virus infection in Brazil: clinical description and implications for health professionals. J. Clin. Microbiol 45, 1370–1372.
de Souza Trindade, G., Li, Y., Olson, V.A., Emerson, G., Regnery, R.L., da Fonseca, F.G., Kroon, E.G., Damon, I., 2008. Real-time PCR assay to identify variants of Vaccinia virus: Implications for the diagnosis of bovine vaccinia in Brazil. J. Virol. Methods 152, 63–71.
Di Giulio, D.B., Eckburg, P.B., 2004. Human monkeypox: an emerging zoonosis. Lancet Infect. Dis. 4, 15–25.
Diniz, S., Trindade, G.S., da Fonseca, F.G., Kroon, E.G., 2001. An outbreak of mousepox in Swiss mice in a laboratory animal facility – case report. Arq. Braz. Med. Vet. Zootec. 53, 152–156.
Donatele, D.M., Travassos, C.E.P.F., Leite, J.A., Kroon, E.G., 2007. Epidemiology bovine poxviruse in the Espírito Santo State. Brazil. Braz.J. Vet. Res. Anim. Sci. 44, 275–282.
Drumond, B.P., Leite, J.A., da Fonseca, F.G., Bonjardim, C.A., Ferreira, P.C., Kroon, E.G., 2008. Brazilian Vaccinia virus strains are genetically divergent and differ from the Lister vaccine strain. Microbes Infect. 10, 185–197.
Duraffour, S., Snoeck, R., de Vos, R., van Den Oord, J.J., Crance, J.M., Garin, D., Hruby, D.E., Jordan, R., De Clercq, E., Andrei, G., 2007. Activity of the anti-orthopoxvirus compound ST-246 against vaccinia, cowpox and camelpox viruses in cell monolayers and organotypic raft cultures. Antivir. Ther. 12, 1205–1216.
Edghill-Smith, Y., Golding, H., Manischewitz, J., King, L.R., Scott, D., Bray, M., Nalca, A., Hooper, J.W., Whitehouse, C.A., Schmitz, J.E., Reimann, K.A., Franchini, G., 2005. Smallpox vaccine-induced antibodies are necessary and sufficient for protection against monkeypox virus. Nat. Med. 11, 740–747.
Emonet, S.F., de la Torre, J.C., Domingo, E., Sevilla, N., 2009. Arenavirus genetic diversity and its biological implications. Infect. Genet. Evol. 9, 417–429.
Erbagci, Z., Erbagci, I., Almila Tuncel, A., 2005. Rapid improvement of human orf (ecthyma contagiosum) with topical imiquimod cream: report of four complicated cases. J. Dermatolog. Treat. 16, 353–356.
Fang, M., Sigal, L.J., 2005. Antibodies and CD8+ T cells are complementary and essential for natural resistance to a highly lethal cytopathic virus. J. Immunol. 175, 6829–6836.
Fenner, F., Wittek, R., Dumbell, K.R. (Eds.), 1989. The Orthopoxviruses. Academic Press, San Diego, California.
Fenner, F., Henderson, D.A., Arita, I., Jezek, Z., Ladnyi, I.D., 1988. Early efforts at control: variolation, vaccination, and isolation and quarantine. In: Smallpox and its Eradication. World Health Organization, Geneva.
Ferreira, J.M., Drumond, B.P., Guedes, M.I., Pascoal-Xavier, M.A., Almeida-Leite, C.M., Arantes, R.M., Mota, B.E., Abrahão, J.S., Alves, P.A., Oliveira, F.M., Ferreira, P.C., Bonjardim, C.A., Lobato, Z.I., Kroon, E.G., 2008a. Virulence in murine model shows the existence of two distinct populations of Brazilian Vaccinia virus strains. PLoS One 3, e3043.
Ferreira, J.M., Abrahão, J.S., Drumond, B.P., Oliveira, F.M., Alves, P.A., Pascoal-Xavier, M.A., Lobato, Z.I., Bonjardim, C.A., Ferreira, P.C., Kroon, E.G., 2008b. Vaccinia virus: shedding and horizontal transmission in a murine model. J. Gen. Virol. 89, 2986–2991.
Fonseca, F.G., Lanna, M.C., Campos, M.A., Kitajima, E.W., Peres, J.N., Golgher, R.R., Ferreira, P.C., Kroon, E.G., 1998. Morphological and molecular characterization of the poxvirus BeAn 58058. Arch. Virol. 143, 1171–1186.
Frandsen, J., Enslow, M., Bowen, A.R., 2011. Orf parapoxvirus infection from a cat scratch. Dermatol. Online J. 17, 9.
Fundação Nacional de Saúde, 2002. Manual de Controle de Roedores, Fundação Nacional de Saúde. Manual de controle de roedores Brasília, Ministério da Saúde, Brazil.
Gammon, D.B., Snoeck, R., Fiten, P., Krecmerova, M., Holy, A., De Clercq, E., Opdenakker, G., Evans, D.H., Andrei, G., 2008. Mechanism of antiviral drug resistance of vaccinia virus: identification of residues in the viral DNA polymerase conferring differential resistance to antipoxvirus drugs. J. Virol. 82, 12520–12534.
Geerinck, K., Lukito, G., Snoeck, R., De Vos, R., De Clercq, E., Vanrenterghem, Y., Degreef, H., Maes, B., 2001. A case of human orf in an immunocompromised patient treated successfully with cidofovir cream. J. Med. Virol. 64, 543–549.
Grosenbach, D.W., Berhanu, A., King, D.S., Mosier, S., Jones, K.F., Jordan, R.A., Bolken, T.C., Hruby, D.E., 2010. Efficacy of ST-246 versus lethal poxvirus challenge in immunodeficient mice. Proc. Natl. Acad. Sci. USA 107, 838–843.
Hammarlund, E., Lewis, M.W., Hansen, S.G., Strelow, L.I., Nelson, J.A., Sexton, G.J., Hanifin, J.M., Slifka, M.K., 2003. Duration of antiviral immunity after smallpox vaccination. Nat. Med. 9, 1131–1137.
Hemmi, H., Kaisho, T., Takeuchi, O., Sato, S., Sanjo, H., Hoshino, K., Horiuchi, T., Tomizawa, H., Takeda, K., Akira, S., 2002. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat. Immunol. 3, 196–200.
Henderson, D.A., 2009. Samllpox the death of a disease. Prometheus Book, New York, pp. 79–105.
Honlinger, B., Huemer, H.P., Romani, N., Czerny, C.P., Eisendle, K., Hopfl, R., 2005. Generalized cowpox infection probably transmitted from a rat. Br. J. Dermatol. 153, 451–453.
Huggins, J., Goff, A., Hensley, L., Mucker, E., Shamblin, J., Wlazlowski, C., Johnson, W., Chapman, J., Larsen, T., Twenhafel, N., Karem, K., Damon, I.K., Byrd, C.M., Bolken, T.C., Jordan, R., Hruby, D.L., 2009. Nonhuman primates are protected from smallpox virus or monkeypox virus challenges by the antiviral drug ST-246. Antimicrob. Agents Chemother. 53, 2620–2625.
Jesus, D.M., Moussatche, N., Damaso, C.R., 2009. In vitro activity of cidofovir against the emerging Cantagalo virus and the smallpox vaccine strain IOC. Int. J. Antimicrob. Agents 33, 75–79.
Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L., Daszak, P., 2008. Global trends in emerging infectious diseases. Nature 451, 990–993.
Jordan, R., Goff, A., Frimm, A., Corrado, M.L., Hensley, L.E., Byrd, C.M., Mucker, E., Shamblin, J., Bolken, T.C., Wlazlowski, C., Johnson, W., Chapman, J., Twenhafel, N., Tyavanagimatt, S., Amantana, A., Chinsangaram, J., Hruby, D.E., Huggins, J., 2009. ST-246 antiviral efficacy in a nonhuman primate monkeypox model: determination of the minimal effective dose and human dose justification. Antimicrob. Agents Chemother. 53, 1817–1822.
Keith, K.A., Hitchcock, M.J., Lee, W.A., Holy, A., Kern, E.R., 2003. Evaluation of nucleoside phosphonates and their analogs and prodrugs for inhibition of orthopoxvirus replication. Antimicrob. Agents Chemother. 47, 2193–2198.
Kulesh, D.A., Baker, R.O., Loveless, B.M., Norwood, D., Zwiers, S.H., Mucker, E., Hartmann, C., Herrera, R., Miller, D., Christensen, D., Wasieloski, L.P., Huggins, J., Jahrling, P.B., 2004. Smallpox and pan-orthopox virus detection by real-time 30 -minor groove binderTaqMan assays on the roche LightCycler and the Cepheid smart Cycler platforms. J. Clin. Microbiol. 42, 601–609.
Kumar, S., Tamura, K., Nei, M., 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform. 5, 150–163.
Lai, A.C., Pogo, B.G., 1989. Attenuated deletion mutants of vaccinia virus lacking the vaccinia growth factor are defective in replication in vivo. Microb. Pathog. 6, 219–226.
Lanier, R., Trost, L., Tippin, T., Lampert, B., Robertson, A., Foster, S., Rose, M., Painter, W., O'Mahony, R., Almond, M., Painter, G., 2010. Development of CMX001 for the Treatment of Poxvirus Infections. Viruses 2, 2740–2762.
Langhammer, S., Koban, R., Yue, C., Ellerbrok, H., 2011. Inhibition of poxvirus spreading by the anti-tumor drug Gefitinib (Iressa). Antiviral Res. 89, 64–70.
Lauterbach, H., Kassub, R., Pätzold, J., Körner, J., Brückel, M., Verschoor, A., Chaplin, P., Suter, M., Hochrein, H., 2010. Immune requirements of post-exposure immunization with modified vaccinia Ankara of lethally infected mice. PLoS One 5, e9659.
Lederman, E.R., Green, G.M., DeGroot, H.E., Dahl, P., Goldman, E., Greer, P.W., Li, Y., Zhao, H., Paddock, C.D., Damon, I.K., 2007. Progressive ORF virus infection in a

patient with lymphoma: successful treatment using imiquimod. Clin. Infect. Dis. 44, e100–e103.
Leite, J.A., Drumond, B.P., Trindade, G.S., Lobato, Z.I., da Fonseca, F.G., dos Santos, J.R., Madureira, M.C., Guedes, M.I., Ferreira, J.M., Bonjardim, C.A., Ferreira, P.C., Kroon, E.G., 2005. Passatempo virus, a vaccinia virus strain. Brazil. Emerg. Infect. Dis. 11, 1935–1938.
Leite, J.A., Drumond, B.P., de Souza Trindade, G., Bonjardim, C.A., Ferreira, P.C., Kroon, E.G., 2007. Brazilian Vaccinia virus strains show genetic polymorphism at the ati gene. Virus Genes 35, 531–539.
Lin, H.Y., Linn, G., Liu, C.B., Chen, C.J., Yu, K.J., 2010. An immunocompromised woman with severe molluscum contagiosum that responded well to topical imiquimod: a case report and literature review. J. Low Genit. Tract. Dis. 14, 134–135.
Lobato, Z.I.P., Trindade, G.S., Frois, M.C.M., Ribeiro, E.B.T., Dias, G.R.C., Teixeira, B.M., Lima, F.A., Almeida, G.M.F., Kroon, E.G., 2005. Outbreak of exantemal disease caused by Vaccinia virus in human and cattle in Zona da Mata Region, Minas Gerais. Arq. Bras. Med. Vet. Zootec. 57, 423–429.
Lopes, O.S., Lacerda, J.P.G., Fonseca, I.E.M., Castro, D.P., Forattini, O.P., Rabelo, E.X., 1965. Cotia virus: a new agent isolated from sentinel mice in São Paulo. Brazil. Am. J. Trop. Med. Hyg. 14, 156–157.
Maiboroda, A.D., 1982. Experimental infection of Norvegian rats (Rattus norvegicus) with ratpox virus. Acta Virol. 26, 288–291.
McLysaght, A., Baldi, P.F., Gaut, B.S., 2003. Extensive gene gain associated with adaptive evolution of poxviruses. Proc. Natl. Acad. Sci. USA 100, 15655–15660.
Megid, J., Appolinário, C.M., Langoni, H., Pituco, E.M., Okuda, L.H., 2008. Vaccinia virus in humans and cattle in southwest region of Sao Paulo state. Brazil. Am. J. Trop. Med. Hyg. 79, 647–651.
Medaglia, M.L., Pessoa, L.C., Sales, E.R., Freitas, T.R., Damaso, C.R., 2009. Spread of cantagalo virus to northern Brazil. Emerg. Infect. Dis. 15, 1142–1143.
Medeiros Silva, D.C., Moreira-Silva, E.A.S., Gomes, J.A.S., da Fonseca, F.G., Correa-Oliveira, R., 2010. Clinical signs, diagnosis, and case reports of Vaccinia virus infections. Braz. J. Infect. Dis. 14, 129–134.
Moss, B., 2007. Poxviridae: The Viruses and Their Replication. In: Knipe, D.M.H., Peter, M. (Eds.), FIELDS Virology, 5th ed. Lippincott Williams & Wilkins, Philadelphia, PA, pp. 2906–2945.
Mota, B.E., Trindade, G.S., Diniz, T.C., da Silva-Nunes, M., Braga, E.M., Urbano-Ferreira, M., Rodrigues, G.O., Bonjardim, C.A., Ferreira, P.C., Kroon, E.G., 2010. Seroprevalence of orthopoxvirus in an Amazonian rural village, Acre. Brazil. Arch. Virol. 155, 1139–1144.
Mota, B.E., Gallardo-Romero, N., Trindade, G., Keckler, M.S., Karem, K., Carroll, D., Campos, M.A., Vieira, L.Q., da Fonseca, F.G., Ferreira, P.C., Bonjardim, C.A., Damon, I.K., Kroon, E.G., 2011. Adverse events post smallpox-vaccination: insights from tail scarification infection in mice with vaccinia virus. PLoS One 6, 18924.
Moussatché, N., Damaso, C.R., McFadden, G., 2008. When good vaccines go wild: Feral Orthopoxvirus in developing countries and beyond. J. Infect. Dev. Ctries. 2, 156–173.
Myskiw, C., Piper, J., Huzarewich, R., Booth, T.F., Cao, J., He, R., 2010. Nigericin is a potent inhibitor of the early stage of vaccinia virus replication. Antiviral Res. 88, 304–310.
Nagasse-Sugahara, T.K., Kisielius, J.J., Ueda-Ito, M., Curti, S.P., Figueiredo, C.A., Cruz, A.S., Silva, M.M., Ramos, C.H., Silva, M.C., Sakurai, T., Salles-Gomes, L.F., 2004. Human vaccinia-like virus outbreaks in São Paulo and Goiás States, Brazil: virus detection, isolation and identification. Rev. Inst. Med. Trop. Sao Paulo. 46, 315–322.
Nitsche, A., Kurth, A., Pauli, G., 2007. Viremia in human Cowpox virus infection. J. Clin. Virol. 40, 160–162.
Neyts, J., Leyssen, P., Verbeken, E., De Clercq, E., 2004. Efficacy of cidofovir in a murine model of disseminated progressive vaccinia. Antimicrob. Agents Chemother. 48, 2267–2273.
Panchanathan, V., Chaudhri, G., Karupiah, G., 2010. Antiviral protection following immunization correlates with humoral but not cell-mediated immunity. Immunol. Cell Biol. 88, 461–467.
Parker, S., Handley, L., Buller, R.M., 2008. Therapeutic and prophylactic drugs to treat orthopoxvirus infections. Future Virol. 3, 595–612.
Quenelle, D.C., Collins, D.J., Kern, E.R., 2003. Efficacy of multiple- or single-dose cidofovir against vaccinia and cowpox virus infections in mice. Antimicrob. Agents Chemother. 47, 3275–3280.
Quenelle, D.C., Collins, D.J., Kern, E.R., 2004. Cutaneous infections of mice with vaccinia or cowpox viruses and efficacy of cidofovir. Antiviral Res. 63, 33–40.
Quenelle, D.C., Prichard, M.N., Keith, K.A., Hruby, D.E., Jordan, R., Painter, G.R., Robertson, A., Kern, E.R., 2007. Synergistic efficacy of the combination of ST-246 with CMX001 against orthopoxviruses. Antimicrob. Agents Chemother. 51, 4118–4124.
Quixabeira-Santos, J.C., Medaglia, M.L., Pescador, C.A., Damaso, C.R., 2011. Animal movement and establishment of vaccinia virus Cantagalo strain in Amazon biome. Brazil. Emerg. Infect. Dis. 17, 726–729.
Reeves, P.M., Bommarius, B., Lebeis, S., McNulty, S., Christensen, J., Swimm, A., Chahroudi, A., Chavan, R., Feinberg, M.B., Veach, D., Bornmann, W., Sherman, M., Kalman, D., 2005. Disabling poxvirus pathogenesis by inhibition of Abl-family tyrosine kinases. Nat. Med. 11, 731–739.
Reeves, P.M., Smith, S.K., Olson, V.A., Thorne, S.H., Bornmann, W., Damon, I.K., Kalman, D., 2011. Variola and monkeypox viruses utilize conserved mechanisms of virion motility and release that depend on abl and SRC family tyrosine kinases. J. Virol. 85, 21–31.
Reiter, M.J., Testerman, T.L., Miller, R.L., Weeks, C.E., Tomai, M.A., 1994. Cytokine induction in mice by the immunomodulator imiquimod. J. Leukoc. Biol. 55, 234–240.
Saijo, M., Ami, Y., Suzaki, Y., Nagata, N., Iwata, N., Hasegawa, H., Ogata, M., Fukushi, S., Mizutani, T., Iizuka, I., Sakai, K., Sata, T., Kurata, T., Kurane, I., Morikawa, S., 2008. Diagnosis and assessment of monkeypox virus (MPXV) infection by quantitative PCR assay: differentiation of Congo Basin and West African MPXV strains. Jpn. J. Infect. Dis. 61, 140–142.
Sbrana, E., Jordan, R., Hruby, D.E., Mateo, R.I., Xiao, S.Y., Siirin, M., Newman, P.C., DA Rosa, A.P., Tesh, R.B., 2007. Efficacy of the antipoxvirus compound ST-246 for treatment of severe orthopoxvirus infection. Am. J. Trop. Med. Hyg. 76, 768–773.
Schatzmayr, H.G., Lemos, E.R., Mazur, C., Schubach, A., Majerowicz, S., Rozental, T., Tânia Maria Pacheco Schubach, T.M.P., Bustamante, M.C., Barth, O.M., 2000. Detection of poxvirus in cattle associated with human cases in the State of Rio de Janeiro: preliminary report. Mem. Inst. Oswaldo Cruz 95, 625–627.
Schatzmayr, H.G., Costa, R.V., Gonçalves, M.C., Barreto, D.F., Batista, V.H., Silva, M.E., Brust, L.A., Barth, O.M., 2009. Human infections caused by vaccinia-like poxviruses in Brazil. Rev. Soc. Bras. Med. Trop. 42, 672–676.
Silva, A.C., Reis, B.B., Ricci Junior, J.E.R., Fernandes, F.S., Corrêa, J.F., Schatzmayr, H.G., 2008. Human infection by the cowpox virus in the microregion of Itajubá, State of Minas Gerais: case report. Rev. Soc. Bras. Med. Tropical 41, 507–511.
Silva-Fernandes, A.T., Travassos, C.E.P.F., Ferreira, J.M.S., Abrahão, J.S., Rocha, E.S.O., Ferreira, F.V., dos Santos, J.R., Bonjardim, C.A., Ferreira, P.C.P., Kroon, E.G., 2009. Natural human infections with Vaccinia virus during bovine vaccinia outbreaks. J. Clin. Virol. 44, 308–313.
Simonetti, B.R., Abreu, D.C., Simonetti, J.P., Gonçalves, M.C.R., Silva, M.E.V., Barth, O.M., Schatzmayr, H.G., 2007. Animal infections by vaccinia-like viruses in the state of Rio de Janeiro:1-Northwestern region. Virus Rev. Res. 12, 32–36.
Skinner Jr., R.B., 2002. Treatment of molluscum contagiosum with imiquimod 5% cream. J. Am. Acad. Dermatol. 47, S221–S224.
Sliva, K., Schnierle, B., 2007. From actually toxic to highly specific–novel drugs against poxviruses. Virol. J. 15, 4–8.
Smee, D.F., Bailey, K.W., Wong, M.H., Wandersee, M.K., Sidwell, R.W., 2004. Topical cidofovir is more effective than is parenteral therapy for treatment of progressive vaccinia in immunocompromised mice. J. Infect. Dis. 190, 1132–1139.
Smith, S.K., Olson, V.A., Karem, K.L., Jordan, R., Hruby, D.E., Damon, I.K., 2009. In vitro efficacy of ST246 against smallpox and monkeypox. Antimicrob. Agents Chemother. 53, 1007–1012.
Stittelaar, K.J., Neyts, J., Naesens, L., van Amerongen, G., van Lavieren, R.F., Holý, A., De Clercq, E., Niesters, H.G., Fries, E., Maas, C., Mulder, P.G., van der Zeijst, B.A., Osterhaus, A.D., 2006. Antiviral treatment is more effective than smallpox vaccination upon lethal monkeypox virus infection. Nature 439, 745–748.
Strenger, V., Muller, M., Richter, S., Revilla-Fernandez, S., Nitsche, A., Klee, S.R., Ellerbrok, H., Zenz, W., 2009. A 17-year-old girl with a black eschar. Cowpox virus infection. Clin. Infect. Dis. 48, 133–134.
Syed, T., Goswami, J., Ahmadpour, O.A., Ahmad, S.A., 1998. Treatment of molluscum contagiosum in males with an analog of imiquimod 1% in cream: a placebo-controlled, double-blind study. J. Dermatol. 25, 309–313.
Tarbet, E., Larson, D., Anderson, B.J., Bailey, K.W., Wong, M.H., Smee, D.F., 2011. Evaluation of imiquimod for topical treatment of vaccinia virus cutaneous infections in immunosuppressed hairless mice. Antiviral Res. 90, 126–133.
Theos, A.U., Cummins, R., Silverberg, N.B., Paller, A.S., 2004. Effectiveness of imiquimod cream 5% for treating childhood molluscum contagiosum in a double-blind, randomized pilot trial. Cutis 74, 134–138.
Testerman, T.L., Gerster, J.F., Imbertson, L.M., Reiter, M.J., Miller, R.L., Gibson, S.J., Wagner, T.L., Tomai, M.A., 1995. Cytokine induction by the immunomodulators imiquimod and S-27609. J. Leukoc. Biol. 58, 365–372.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.
Trindade, G.S., da Fonseca, F.G., Marques, J.T., Diniz, S., Leite, J.A., De Bodt, S., Van der Peer, Y., Bonjardim, C.A., Ferreira, P.C., Kroon, E.G., 2004. Belo Horizonte virus: a vaccinia-like virus lacking the A-type inclusion body gene isolated from infected mice. J. Gen. Virol. 85, 2015–2021.
Trindade, G.S., Lobato, Z.I., Drumond, B.P., Leite, J.A., Trigueiro, R.C., Gustavo, M.I., da Fonseca, F.G., dos Santos, J.R., Bonjardim, C.A., Ferreira, P.C.P., Kroon, E.G., 2006. Short report: Isolation of two vaccinia virus strains from a single bovine vaccinia outbreak in rural area Brazil: Implications on the emergence of zoonotic orthopoxvirus Am. J. Trop. Med. Hyg. 75, 486–490.
Trindade, G.S., Emerson, G.L., Carroll, D.S., Kroon, E.G., Damon, I.K, 2007a. Brazilian vaccinia viruses and their origins. Emerg. Infect. Dis. 13, 965–972.
Trindade, G.S., Vilela, J.M., Ferreira, J.M., Aguiar, P.H., Leite, J.A., Guedes, M.I., Lobato, Z.I., Madureira, M.C., da Silva, M.I., da Fonseca, F.G., Kroon, E.G., Andrade, M.S., 2007b. Use of atomic force microscopy as a diagnostic tool to identify orthopoxvirus. J. Virol. Methods 141, 198–204.
Trindade, G.S., Guedes, M.I., Drumond, B.P., Mota, B.E., Abrahão, J.S., Lobato, Z.I., Gomes, J.A., Corrêa-Oliveira, R., Nogueira, M.L., Kroon, E.G., da Fonseca, F.G., 2009. Zoonotic vaccinia virus: clinical and immunological characteristics in a naturally infected patient. Clin. Infect. Dis. 48, e37–e40.
Toutous-Trellu, L., Hirschel, B., Piguet, V., Schiffer, V., Saurat, J.H., Pechère, M., 2004. Treatment of cutaneous human papilloma virus, poxvirus and herpes simplex virus infections with topical cidofovir in HIV positive patients. Ann. Dermatol. Venereol. 131, 445–449.

Tulman, E.R., Delhon, G., Afonso, C.L., Lu, Z., Zsak, L., Sandybaev, N.T., Kerembekova, U.Z., Zaitsev, V.L., Kutish, G.F., Rock, D.L., 2006. Genome of horsepox virus. J Virol. 80, 9244–9258.

Tzahar, E., Moyer, J.D., Waterman, H., Barbacci, E.G., Bao, J., Levkowitz, G., Shelly, M., Strano, S., Pinkas-Kramarski, R., Pierce, J.H., Andrews, G.C., Yarden, Y., 1998. Pathogenic poxviruses reveal viral strategies to exploit the ErbB signaling network. EMBO J. 17, 5948–5963.

Valadares, Y.M., Brandao, G.C., Kroon, E.G., Filho, J.D., Oliveira, A.B., Braga, F.C., 2009. Antiviral activity of *Solanum paniculatum* extract and constituents. Z. Naturforsch. C 64, 813–818.

Vestergaard, L., Vinner, L., Andersen, K.E., Fomsgaard, A., 2008. Identification of cowpox infection in a 13-year-old Danish boy. Acta Derm. Venereol. 88, 188–190.

Vigne, S., Germi, R., Duraffour, S., Larrat, S., Andrei, G., Snoeck, R., Garin, D., Crance, J.M., 2008. Specific inhibition of orthopoxvirus replication by a small interfering RNA targeting the D5R gene. Antivir. Ther. 13, 357–368.

Vigne, S., Duraffour, S., Andrei, G., Snoeck, R., Garin, D., Crance, J.M., 2009. Inhibition of vaccinia virus replication by two small interfering RNAs targeting B1R and G7L genes and their synergistic combination with cidofovir. Antimicrob. Agents Chemother. 53, 2579–2588.

Vora, S., Damon, I., Fulginiti, V., Weber, S.G., Kahana, M., Stein, S.L., Gerber, S.I., Garcia-Houchins, S., Lederman, E., Hruby, D., Collins, L., Scott, D., Thompson, K., Barson, J.V., Regnery, R., Hughes, C., Daum, R.S., Li, Y., Zhao, H., Smith, S., Braden, Z., Karem, K., Olson, V., Davidson, W., Trindade, G., Bolken, T., Jordan, R., Tien, D., Marcinak, J., 2008. Severe eczema vaccinatum in a household contact of a smallpox vaccinee. Clin. Infect. Dis. 46, 1555–1561.

Wagner, T.L., Ahonen, C.L., Couture, A.M., Gibson, S.J., Miller, R.L., Smith, R.M., Reiter, M.J., Vasilakos, J.P., Tomai, M.A., 1999. Modulation of TH1 and TH2 cytokine production with the immune response modifiers, R-848 and imiquimod. Cell Immunol. 191, 10–19.

Wilson, D.E., Reeder, D.M., 2005. Mammal Species of the World: A Taxonomic and Geographic Reference, 3rd ed. Johns Hopkins University Press, Baltimore, Maryland, pp. 2.142.

Xu, R., Johnson, A.J., Liggitt, D., Bevan, M.J., 2004. Cellular and humoral immunity against vaccinia virus infection of mice. J. Immunol. 172, 6265–6271.

Yang, G., Pevear, D.C., Davies, M.H., Collett, M.S., Bailey, T., Rippen, S., Barone, L., Burns, C., Rhodes, G., Tohan, S., Huggins, J.W., Baker, R.O., Buller, R.L., Touchette, E., Waller, K., Schriewer, J., Neyts, J., DeClercq, E., Jones, K., Hruby, D., Jordan, R., 2005. An orally bioavailable antipoxvirus compound (ST-246) inhibits extracellular virus formation and protects mice from lethal orthopoxvirus challenge. J. Virol. 79, 13139–13149.