HUMAN VACCINIA-LIKE VIRUS OUTBREAKS IN SÃO PAULO AND GOIÁS STATES, BRAZIL: VIRUS DETECTION, ISOLATION AND IDENTIFICATION

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SUMMARY

Since October 2001, the Adolfo Lutz Institute has been receiving vesicular fluids and scab specimens of patients from Paraíba Valley region in the São Paulo and Minas Gerais States and from São Patricio Valley, in the Goiás State. Epidemiological data suggested that the outbreaks were caused by Cowpox virus or Vaccinia virus. Most of the patients are dairy milkers that had vesiculo-pustular lesions on the hands, arms, forearms, and some of them, on the face. Virus particles with orthopoxvirus morphology were detected by direct electron microscopy (DEM) in samples of 49 (66.21%) patients of a total of 74 analyzed. Viruses were isolated in Vero cell culture and on chorioallantoic membrane (CAM) of embryonated chicken eggs. Among 21 samples submitted to PCR using primers for hemagglutinin (HA) gene, 19 were positive. Restriction digestion with TaqI resulted in four characteristic Vaccinia virus fragments. HA nucleotide sequences showed 99.9% similarity with Cantagalo virus, described as a strain of Vaccinia virus. The only difference observed was the substitution of one nucleotide in the position 616 leading to change in one amino acid of the protein in the position 206. The phylogenetic analysis showed that the isolates clustered together with Cantagalo virus, other Vaccinia strains and Rabbitpox virus.

KEYWORDS: Vaccinia-like virus; Outbreak; Diagnosis; Electron microscopy; Virus isolation; PCR; Sequencing; Phylogenetic analysis.

INTRODUCTION

Poxviruses, measuring about 200-350 x 115-260 nm, are the largest known viruses being on the limit of the optical microscopy resolution. They present complex capsid symmetry and cores containing nucleic acids, surrounded by multiple membranes originating brick-shaped or oval forms. Two morphologic forms are described by negative staining electron microscopy, the “M” form that presents rough surface similar to the mulberry and the “C” one, with a capsule and smooth surface. They comprise a large family of linear double-stranded DNA viruses of approximately 200 kbp and the replication takes place in the host cell cytoplasm, which is different from other DNA viruses where replication occurs inside the cell nucleus.

The genus Orthopoxvirus belongs to the sub-family Chordopoxvirinae, some of which are pathogens of human and veterinary importance, causing zoonosis of impact to the public health and local economic impairment. The main orthopoxviruses that infect humans are: the already eradicated Variola virus; Monkeypox virus, responsible for a recent outbreak in the USA; Cowpox virus, described in Europe and Vaccinia, a virus of uncertain origin reported as the causal agent of outbreaks in India, and which was used in the vaccination campaign for smallpox eradication. The Cowpox virus and Vaccinia virus cause a similar, localized lesion in humans when in contact with infected cow udders.

The classic diagnosis for Orthopoxvirus was accomplished by virus isolation on chorioallantoic membrane (CAM) of embryonated chicken eggs and in susceptible cell cultures, detection of virus particles by electron microscopy using negative staining method and by serological methods. Currently, molecular biology techniques such as the polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) and sequencing of viral genome are the chosen methods for the identification and characterization of these viruses.

From October 2001 to January 2003 the Adolfo Lutz Institute (São Paulo, Brazil) received specimens of vesicular fluids and scabs from...
patients with clinical suspicion of “vesicular stomatitis” or “bovine variola”. These patients were from the rural area of Paraíba Valley counties, at the East side of São Paulo State. Most of them were dairy milkers that presented lesions with vesiculo-pustular aspect on the hands, arms, forearms and some of them, in the face. In May 2003, we received the same kind of samples from São Patricio Valley, central area of Goiás State.

Our objective is to report human outbreaks caused by a Vaccinia-like virus occurring in different regions of Brazil. After virus detection by DEM, we have isolated the virus on CAM of embryonated chicken eggs and in Vero cell culture, and identified it by molecular biology techniques. We emphasize the necessity of keeping our skillness in applying the old classical methodology for viral diagnosis together with the new advanced molecular techniques so as to be able to identifying the new emergent and reemergent viral agents.

MATERIAL AND METHODS

1. Patients and biological specimens: Specimens of vesicular fluids and skin lesion crusts of 74 patients were received from October 2001 to July 2003. Sixty-eight (92%) were from 10 counties of Paraíba Valley in São Paulo State; two (2.7%) from Minas Gerais State and four (5.4%) from São Patricio Valley, Goiás State (Table 1). The health authorities of Goiás State had notified 18 cases, but samples of only four patients had been sent to us. The distance between the two localities is approximately 1,200 km. The conditions of the collection, storage and consignment of the specimens to the laboratory were not in agreement with the required norms for viral diagnosis. Glass slides with smears of vesicular fluids and scabs were received without refrigeration, wrapped up in aluminum foil. The number of samples varied from one to three per patient. Samples received in triplicate were also submitted for molecular biology tests and virus isolation. Thus, for molecular characterization, 21 smear samples from different counties were processed and, to evaluate some biological aspects, 12 samples were inoculated on CAM of embryonated chicken eggs and in cell culture.

2. Direct electron microscopy (DEM): The original biological specimens were resuspended in 50 µL of phosphate-buffered saline (PBS) pH 7.2. One drop of the suspension was put on EM grid and submitted to negative staining technique6 with 2% potassium phosphotungstate (PTK) pH 6.4. The grids were examined and the viruses were documented in a Philips EM400-T electron microscope operating in 80 kV. All 74 patients had samples examined by EM.

3. Virus isolation

3.1 Embryonated chicken eggs: Before inoculation into embryonated eggs and cell culture, 12 samples were each resuspended in 500 µL 0.01M PBS pH 7.2 containing penicillin (500U) and streptomycin (500 µg/mL) (Sigma, St. Louis, USA). The suspensions, diluted to 1:5 and 1:15 in PBS containing antibiotics were inoculated on 10-12 days CAM of embryonated chicken eggs. The embryos were candled and the inoculation site was marked with a pencil. Each egg was inoculated with 200 µL of each dilution directly into the CAM using a disposable tuberculin syringe. The inoculated eggs were incubated at 37 ºC for 72 hours.

3.2 Cell culture: The same initial viral suspensions used on embryonated chicken eggs were inoculated in cell culture. Vero cells (ATCC CCL-81) in culture tubes containing 2x10⁵ cells/mL were grown in medium 199 and 0.1 mM non-essential amino acids, supplemented with 10% inactive fetal calf serum (FCS). The confluent cells were inoculated with 200 µL of each sample suspension. After one hour at room temperature, 1 mL of medium with 2% FCS was added to each inoculated tube and they were incubated at 37 ºC. The medium was replaced every three days. Cell cultures were observed for CPE daily during seven days. The cells with CPE were washed with PBS, fixed in situ with 1% glutaraldehyde in PBS and consigned to the laboratory for electron microscopy.

Table 1

Results of EM, PCR and virus isolation in cell culture and on CAM, according to the localities of the received samples

<table>
<thead>
<tr>
<th>County - Region - State (number of cases)</th>
<th>EM</th>
<th>PCR</th>
<th>Cell culture</th>
<th>CAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Areias - Paraíba Valley - SP (03)</td>
<td>1/3</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Cachoeira Paulista - Paraíba Valley - SP (06)</td>
<td>6/6</td>
<td>3/3</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Canas - Paraíba Valley - SP (05)</td>
<td>3/5</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Cruzeiro - Paraíba Valley - SP (05)</td>
<td>3/5</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Guaratinguetá - Paraíba Valley - SP (03)</td>
<td>2/3</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Lagoinhas - Paraíba Valley - SP (16)</td>
<td>8/16</td>
<td>3/4</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Lavrinhas - Paraíba Valley - SP (03)</td>
<td>2/3</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Lorena - Paraíba Valley - SP (01)</td>
<td>0/1</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Piquete - Paraíba Valley - SP (02)</td>
<td>2/2</td>
<td>1/2</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Silveiras - Paraíba Valley - SP (24)</td>
<td>18/24</td>
<td>7/7</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Delfim Moreira - Paraíba Valley - MG (01)</td>
<td>0/1</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Juiz de Fora - MG (01)</td>
<td>0/1</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Rianópolis - São Patricio Valley - GO (03)</td>
<td>3/3</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Morro Agudo - São Patricio Valley - GO (01)</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Total</td>
<td>49/74</td>
<td>19/21</td>
<td>12/12</td>
<td>12/12</td>
</tr>
</tbody>
</table>

NT = not tested
0.15M PBS pH 7.2 for one hour at 4 °C. After being rinsed twice with cacodylate buffer and stained directly with toluidine blue the cells were examined by light microscopy and photographed.  

4. Molecular biology techniques: The molecular biology techniques (PCR, sequencing, RFLP, similarity determination of the sequences and phylogenetic analyses) had the purpose of identifying the virus detected by EM.

4.1 Viral DNA extraction: Viral DNA was obtained directly from 21 smears samples, one Vero cell culture isolate and one positive control sample (Vac - Ist - strain Elstree - Liverpool - UK) using a modified protocol described by ROPP et al. Briefly, the digested DNA was extracted twice with phenol-chloroform-isooamyl alcohol solution (25:24:1) (Sigma, St. Louis, MO), precipitated with isopropanol, washed with 70% ethanol, air dried and dissolved in 50 µL of milliQ water.

4.2 Primers: The primers EACP1 (ATGACACGATTGCCAATAC) and EACP2 (CTAGACATTGTTTTCTG) described by ROPP et al. were used to amplify HA gene of Eurasia-African subgroup of the genus Orthopoxvirus, producing amplicons of 927 bp. We also used the set of primers HAOUTF (CCAAATATATTCCCATAGTC), HAOUTR (GACATAATCTATCTGGATC), HAINTF (GACATAATATCTCTGGATC) and HAINTR (GTGGATTCTTCAGATGATGC), described by DAMASO et al. The primers HAOUTF and HAOUTR flank the open reading frame of the HA gene amplifying a product of 1180 bp which includes the 927 nucleotides of the HA gene. The pair of primers HAINTF and HAINTR, which anneal in the mid-region of the HA coding sequence, was used in the sequencing reaction to provide overlapping sequences.

4.3 Polymerase chain reaction (PCR): PCR in a total volume of 100 µL was performed in the following conditions: 5 µL of the DNA sample; 25 pmol of each primer; 20 mM tris-HCl (pH 8.4); 50 mM KCl; 1.5 mM MgCl2; 0.25 mM of each dNTPs; 2U Taq DNA polymerase (Invitrogen, Carlsbad, CA). The used program started with a denaturation step at 94 °C for one min, annealing at 50 °C for one min and polymerization at 72 °C for one min, ending with a step at 72 °C for five min, using a 2400 Perkin Elmer Cetus thermal cycler. The same PCR reagents and protocol were used to the set of primers EACP1/EACP2 and HAOUTF/HAOUTR.

PCR products were identified by electrophoresis in 1% agarose gel containing 0.5 µg/mL ethidium bromide (Sigma, St. Louis, MO) in 40 mM tris-acetate and 1 mM EDTA buffer (TAE) (Sigma, St. Louis, MO).  

4.4 Restriction fragment length polymorphism (RFLP): Restriction digestions were done adding 5U of TaqI (MBI Fermentas, Hanover, MD) in 30 µL of DNA amplified with EACP1/EACP2 primers and incubated at 65 °C for two hours. DNA products were fractionated by gel electrophoresis in 2% agarose gel in TAE buffer.

4.5 HA gene sequencing: Twelve PCR amplified samples from seven different counties (Cachoeira Paulista, Guaratinguetá, Lagoimina, Lorena, Piquete, Silveiras and Rianópolis) were sequenced.

The amplicons were purified with the commercial kit “Concert rapid PCR extraction system” (GIBCO/BRL, Carlsbad, CA) following the procedure described by the manufacturer. About 20 ng of the purified amplicon were sequenced with four primers (HAOUTF, HAOUTR, HAINTF and HAINTR) using the kit “BIGDYE Terminator Cycle Sequencing” (Foster City, CA). An automated DNA sequencer, model 377 ABI PRISM, Applied Biosystems, Inc. was used.

The obtained sequences were edited in the Sequence Navigator 1.0 program (Applied Biosystems, Foster City, CA). The comparative analyses of the DNA sequences were based on nucleotide and amino acid aligned sequences using CLUSTAL X software. The phylogenetic tree, based on aligned nucleotides, was constructed, applying the neighbor-joining method with calculated bootstrap confidence intervals of 1000 heuristic search replicate, using MEGA, version 2.1 (Molecular Evolutionary Genetics Analyses, Pennsylvania State University). Genetic distance was determined by proportional distance method, Kimura two parameter model, applying pairwise deletion of gaps and equally weighting both transition and transversion for all three codon positions.

To determine the similarity among the 12 HA gene nucleotide sequences and the Cantagalo virus it was used the module MegaAlign 4.00 of the program DNASTAR (software Lasergene).

RESULTS

1. Patients: Sixty-six patients were male, eight female and the age ranged from 12 to 78 years old (Fig. 1). Paraíba Valley region has in the milk production and dairy products, one of its main economic activities. The majority of the patients were dairymaids, milkmen and some were farmers and their relatives. São Patricio Valley comprises many farms of vast extension dedicated to beef cattle production. As clinical characteristic, they presented skin lesions in the form of vesicles with festering secretion, mainly on the hands, fingers, arms and on the face. Other clinical symptoms were fever, sweat, headache, appetite loss, erythema, pains in the body, itches in the lesion and increased axillary ganglions. Three patients had to be taken into hospital because they developed more serious symptoms.

![Fig. 1 - Number of cases and positive cases by EM according to age range.](image-url)
2. Electron microscopy: Virus particles with characteristic morphology of orthopoxvirus (Fig. 2) were detected in 66.21% (49/74) of the examined samples. Out of 49 positive samples, 45 (91.8%) were from patients of Paraíba Valley-SP and four (8.2%) of São Patricio-GO. The two samples from Minas Gerais State were negative (Table 1). The viruses had brick-shaped form, measuring about 310 x 245 nm (Fig. 2) with dumbbell-shaped core. Most of virus presented “M” form morphology, with rough surface formed by filaments (Fig. 2a). Morphology of “C” form particles was also observed, however in smaller number (Fig. 2b). Isolated viral particles were predominant but some small agglomerates were also observed (Figs. 2c and 2d). About 70% of the positive samples presented a great number of viral particles.

3. Virus isolation

3.1 CAM of embryonated chicken eggs: All the 12 samples inoculated on CAM of embryonated chicken eggs resulted positives. The viruses growth on CAM produced large pocks with 3 to 4 mm diameter, central necrosis and ulcerations with slightly hemorrhagic appearance (Fig. 3c). The presence of the virus in these pocks was confirmed by EM.

3.2 Cell culture: The 12 samples inoculated in cell culture were also positives (Table 1). The first alterations in infected cells were observed 24 hours post-inoculation, consisting in increased refractability and rounding cells. After seven days, cytopathic effects (CPE) consisting of multinucleated cell plaques with extensive degeneration was observed (Figs. 3a e 3b).

On the second passage, CPE became detectable on the second day. Orthopoxvirus particles were detected by EM in the second passage of the cell culture sediment and confirmed by PCR.

4. Analyses by molecular biology techniques

4.1 PCR amplification: PCR with EACP1 and EACP2 primers produced an amplicon of 927 bp, in agreement with the results obtained by ROPP et al.28. The primers HAOUTF and HAOUTR, described by DAMASO et al., amplified a product of 1180 bp, compatible with the expected for Orthopoxvirus. The Fig. 4 shows PCR results of one sample (Silveiras-2382) and the positive control (Vac - lst - Elstree - Liverpool - UK) with the two sets of primers. During the standardization phase of PCR, the DAMASO’s primers resulted in better yield of amplification. Therefore, we decided to use these primers for all PCRs (21 patient samples, 1 Vero cell culture isolate and positive control), except for RFLP, where it was used ROPP’s primers. Of the 21 samples submitted to PCR, 19 were positive. The Vero cell culture isolate was also positive.

4.2 Sequencing: The result of the HA nucleotides alignment of the 12 samples and the Cantagalo virus indicates a similarity of 99.9%
between them. The only difference was the change of nucleotide A to G in the position 616 of HA gene (Fig. 5a), resulting in substitution of the amino acid Lysine-K (basic character) for the glutamic acid-E (acid character) in the position 206 (Fig. 5b). As the 12 sequenced samples were identicals, one of them (Goiás 2549) was selected to represent the group (Figs. 5a e 5b).

4.3 RFLP: The RFLP of the amplified 927 bp products of the HA gene resulted in four fragments (451, 295, 105 and 97 bp) as expected, and in agreement with the results obtained by ROPP et al.28 (data not shown). Moreover, the nucleotide sequencing analysis of the amplicon confirmed the presence of 3 *Taq*I restriction sites (Fig. 5a).

4.4 Phylogenetic analysis: The phylogenetic analysis of the HA gene shows that our samples, represented by one of each county, clustered together with *Cantagalo*, *Rabbiptox* and *Vaccinia virus* (Fig. 6).

**DISCUSSION**

Since smallpox eradication in the end of the 70s, reports on outbreaks of human infections by Poxvirus have been rare^33^. Different viruses such as *Cowpox virus* and *Vaccinia virus* (genus *Orthopoxvirus*); *Orf, Bovine Papular Stomatitis and Pseudocowpox* (genus *Parapoxvirus*); and *Bovine Herpes 2* (Herpesviridae) can cause very similar lesions in cow udders and in dairymaid hands and arms^4,45^.

The etiological diagnosis of these diseases, commonly known as “disease of dairymaid”, “bovine variola”, “pseudovariola” or still “paravaccinia” is difficult to be established correctly. According to WEIBLEN^46^, cases clinically diagnosed as “pseudovariola” are not always confirmed at the laboratory as *Parapoxivirus*. Outbreaks initially attributed to *Cowpox virus* were later on confirmed to be caused by *Vaccinia virus*^21^.

In our case, the available clinical and epidemiological data, plus the EM results, have suggested that a Vaccinia-like virus or even a Cowpox-like virus not yet described in our country was the pathogen associated with these outbreaks.

Although it is not possible to distinguish the species of the genus *Orthopoxvirus*, DEM is the easiest and quickest laboratory diagnostic method available to differentiate morphologically these viruses from *Parapoxvirus* and *Herpesviridae*, making this technique an important
The great number of human cases analyzed is expressive mainly in Paraíba Valley (Table 1). We could not find any published report on outbreaks of Variola virus with so many cases. It is probable that manual milking, still a very common practice in that region by small producers, the proximity of the farms and the trade facility in cattle fairs have facilitated the widespread of the virus. We have to mention that at the same occasion there was a great “bovine variola” outbreak that affected about 1,500 cattle in the same region (Paraíba Valley)\(^{19,40,41}\). The other region where an outbreak occurred is São Patricio Valley, approximately 1,200 km far from Paraíba Valley (Fig. 7). Between both localities there are many small cities and geographic accidents.

The disease affected people of all age groups, with predominance in the groups below 30 years of age (Fig. 1). This is an expected result because most of dairy workers in these groups were not vaccinated against smallpox and they are the ones that have closer contact with the cattle. Most of the human infections by Cowpox virus\(^{4,18}\) and Monkeypox virus\(^{26}\) also occur in individuals below 18 years old that were not vaccinated against smallpox. Seroepidemiology studies detected a high prevalence of total and neutralizing antibodies in the population above 30 years old, which have been vaccinated against smallpox\(^{2,25}\). In 1979, after two years without any registration of smallpox case following the successful worldwide vaccination campaign, the WHO proclaimed that the disease had been eradicated from the World.

In spite of we could not have sequenced all samples, the analyzed group can be considered representative because possess samples of all affected areas. The sequencing and profile analyses after digestion with TaqI of the 927 bp PCR product of samples from counties of São Paulo and Goiás States, showed that the isolates were Vaccinia-like, similar to the Cantagalo virus\(^3\). The distinction between Variola virus and Cowpox virus can be done easily with the restriction enzyme TaqI which recognize the palindromic TCGA of the gene HA, generating four fragments in the case of Variola virus and six in the Cowpox virus\(^2\). The sequence analyses of the amplitudes revealed the presence of three restriction sites, resulting in four fragments of sizes compatible to those described by ROPP et al.\(^{23}\) (Fig. 5a). The nucleotides sequence of this isolated strain, compared with that of Cantagalo virus showed only one nucleotide alteration, in the position 616 (Fig. 5a), resulting in substitution of the amino acid Lysine-K (basic character) for the glutamic acid-E (acid character) (Fig. 5b).

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Two strains of Poxvirus, SPAn-232 and BeAn-58058, isolated in Brazil in the 1960s, have been characterized only recently by molecular biology techniques. The virus SPAn-232, isolated from a sentinel mouse in the rural area of Cotia-SP, was characterized as a Vaccinia-like virus and, maybe, it could have some relation to all Vaccinia-like viruses that now are being detected in our country. Even though the vector of that virus is not known, the fact that the virus has been isolated from a sentinel mouse, suggests that it could be an arthropod. The BeAn-58058, isolated from wild rodents in surrounding areas of Belém-PA, also presents molecular aspects similar to the Vaccinia-like virus. The comparison of sequences of these two viruses with the recent isolates could explain many epidemic aspects. The accomplishment of a seroepidemiologic study would also be important to evaluate the prevalence of specific antibodies in the population. The antibody levels in human population, in cattle and in wild animals would bring important informations about the mechanisms of infection by those viruses, as well as would allow the evaluation of the population’s protection level against Variola virus, which underwent bioengineering treatment to become a powerful bioweapon against human population.

The endemic character of this disease caused by Vaccinia-like virus in some regions of Brazil seems to be established. There are few studies about Poxviruses in Brazil and with this report is intended to point the need of improving the epidemiologic surveillance with a seroepidemiologic study that would help to determine the transmission chain, as well as to identify the natural host of those viruses, once this zoonosis is detrimental to the local economy besides being a public health problem.

Surtos de vírus Vaccinia-like nos Estados de São Paulo e Goiás, Brasil: detecção, isolamento e identificação viral

A partir de outubro de 2001, o Instituto Adolfo Lutz tem recebido amostras de lâmina vesicular e crostas de lesões de pele de pacientes das regiões do Vale do Paraíba, Estado de São Paulo e do Vale do São Patricio, Estado de Goiás. Os dados clínicos e epidemiológicos sugeriram que os surtos poderiam ser causados por Cowpox virus ou Vaccinia virus. A maioria dos pacientes era ordenhadores que tinham lesões vesiculopustulares nas mãos, braços, antebraços e alguns na face. A análise por microscopia eletrônica direta (MED) detectou partículas com morfologia de vírus do gênero Orthopoxviruses em amostras de 49 (66,21%) pacientes dos 74 analisados. Os vírus foram isolados em membrana corioalantoide (MCA) de ovo embrionado de galinha e em linhagem celular Vero com confirmação por MED e PCR. Das 21 amostras de lesões submetidas ao PCR utilizando iniciadores para o gene da hemaglutinina (HA), 19 foram positivas. A digestão por enzima de restrição TaqI resultou em quatro fragmentos característicos de Vaccinia virus. A análise nucleotidica do sequenciamento revelou que esses vírus apresentam 99,9% de similaridade com o Cantagalo virus, descrito como uma cepa de Vaccinia virus, havendo apenas alteração de um nucleotídeo na posição 616 com mudança de um aminocárdido na proteína na posição 206. A análise filogenética mostrou que os isolados se agruparam junto aos Cantagalo virus, outras cepas de Vaccinia virus e Rabbitpox virus.

ACKNOWLEDGEMENTS

We thank Drs. Hatune Tanaka, Dalton Ramalho Weigl, Eliseu Alves Waldman, Júlia Maria Martins de Souza Felippe and Zoraida C. F. Grilli for a critical review of this paper and suggestions. We also thank Dr. João Luiz Costa Cardoso (Instituto Butantan) for kindly providing the Cantagalo virus for vaccinia antibodies in staff members of Bio-Manguinhos/Fiocruz. In: BRIT. J. DERM., 131: 598-607, 1994.

REFERENCES


