AN RT-NESTED-PCR FOR DIAGNOSIS OF ALAGOAS VESICULOVIRUS INFECTION IN GUINEA PIGS

ABSTRACT

Guinea pigs were inoculated by the intradermal route into the tarsal pad with Indiana 3-Alagoas virus (Rhabdoviridae, Vesiculovirus) suspension. The animals exhibited vesicular lesions in the tarsal pad with generalization to others pads and mouth three days after infection. After the seventh serial passages, the virus presented a suitable adaptation to infection in guinea pigs. Ten blood and tarsal pad epithelial samples of the infected guinea pigs showed that the Vesiculovirus genome could be amplified by RT-nested-PCR. The data obtained with this technique demonstrated that it might be suitable for diagnosis of veterinarian and human Vesiculovirus infections.

INTRODUCTION

The genus Vesiculovirus of the Rhabdoviridae family comprises enveloped bullet shaped 180nm long virus particles. The well characterized viruses are of the serotypes New Jersey, Indiana, Indiana 2 (Cocal), Indiana 3 (Alagoas), Jurona, Carajas, Maraba, Piry, Calchaque, Yug Bogdanovac, Isfahan, Chandipura, Perinet, Porton-S (Shope & Tesh 1987). The Brazilian viruses Maraba and Carajas remain ungrouped in the Indiana serotype (Travassos da Rosa et al. 1984).

Vesiculovirus infection of vertebrate and invertebrate animals and plants are worldwide distributed in nature. New Jersey and Indiana 1 serotypes and subtypes, Indiana 2 (Cocal and Argentina strains viruses) and Indiana 3 (Alagoas, Brazil strain) (Federer et al. 1967) are important because they are the causal agent of vesicular stomatitis (VS), a disease of cattle, horses and swine. The disease is characterized by vesicular lesions on the mucous membrane of the tongue, lips, cheeks, gums, palate, teats, and the hooves, and its symptoms resemble those of foot-and-mouth disease (FMD). In Brazil, the VS virus (Indiana 3-Alagoas) was first isolated and identified in the State of Alagoas in 1964, and the Indiana 2-Cocal in the State of SãoPaulo in 1966 (Federer et al. 1967, Pustiglione et al. 1987, Andrade 1974, Andrade et al. 1980). Piry, Chandipura and Isfahan viruses produce human acute febrile illness and meningoen-

Piry, Maraba and Carajas viruses are common causatives of human infection in Brazil. Serologic surveys carried out in Brazilian Northeastern and Southeastern inland towns showed that 16% and 14.3% of the participants presented neutralizing antibodies to Piry virus, respectively (Tavares Neto 1992). In a serologic survey carried out in equines in 1974, 25.2% of the animals, from different Brazilian regions, showed antibodies to Indiana 3-Alagoas virus (Andrade 1974). In the present study, using guinea pigs infected with Indiana 3-Alagoas virus strain, we showed the usefulness of the RT-nested-PCR for detection of veterinary and human Vesiculovirus in clinical samples.

**MATERIAL AND METHODS**

Indiana 3-Alagoas virus Br/64, kindly supplied by Dr. Cláudio Andrade from the Veterinary School of the Universidade Federal Fluminense, Niterói, Brazil, was used for the study.

Guinea pigs (*Cavia porcellus*) were inoculated by the intradermal route into the tarsal pad with the supernatant fluid of C6/36 (*Aedes albopictus*) tissue culture infected with the Indiana 3–Alagoas virus strain, using an insulin subcutaneous injection syringe. After nearly six to seven serial passages the virus presented a suitable adaptation to the guinea pigs, which exhibited vesicular lesions in the tarsal pad, and generalization to other pads and mouth, three days after infection.

In sequence, the vesicle fluid of the lesions in the infected pads of guinea pigs were spread by intradermal route on the tarsal pads of a group of two dozen adult guinea pigs. The animals were observed daily. Blood and tissues were collected from the tarsal pad lesions. All animals developed generalized lesions.

RNA was extracted from blood and epithelial tissue of the tarsal pads of the infected guinea pigs using the Qiamp DNA Blood Mini kit (Quiagen, USA). A reverse transcription followed by polymerase chain reaction (RT-PCR) was performed in the same tube in order to confirm the Alagoas virus infection in the animals. For the test, transcription and the PCR were carried out in the same tube. The reaction mixture included 5μL of each RNA extract, 0.3mM of *Vesiculovirus* G complementary primer (5'–CAGATGGTATGGACCCAAATA-3'), 4.5μL of a Ca and Mg buffer, 0.1M dNTPs, 10U of RNAase inhibitor and 10U reverse transcriptase. The reaction mixture was incubated at 41°C for 1h. For the PCR, 0.3μM of *Vesiculovirus* G sense primer (5'–CCACACCGATGAATTGGAC-3') and *Vesiculovirus* G reverse primer, 1U of Taq DNA polymerase, 0.3μM of primer sense and reverse and 50μL of H₂O DEPC were added to the tube. The mixture was submitted to 40 cycles at 93°C for 90sec, 50°C for 2min and 72°C for 4min.

Following, a nested-PCR was used for confirmation of the *Vesiculovirus* origin of amplicons obtained by RT-PCR. The reaction mixture contained 1μL of the RT-PCR amplicon, 1U of Taq DNA polymerase (Pharmacia, USA), 5μL of buffer 10×, 0.3mM of Piry complementary (5'–CATCTGAGACCGACAACATC3') and sense (5'TCATTGGACCAACATGCC3') internal primers (these primers were selected based on the Piry virus G gene nucleotide sequence) in a total volume of 50μL. The mixture was submitted to 35 cycles at 93°C for 90sec, 50°C for 2min and 72°C for 2min.

Amplicons obtained by RT-PCR and nested-PCR were submitted to an electrophoresis in 1.7% agarose gel stained with ethidium bromide, visualized under UV light. The size of the amplicons was determined by comparison with a 100 bp DNA ladder (Promega, USA).

**RESULTS**

The guinea pigs infected with Indiana 3–Alagoas virus presented vesicular lesions with flogistic signs in the epithelial tissue of the tarsal pads two days after inoculation, as shown in Figure 1. Animals inoculated with uninfected tissue culture fluid (negative control) did not show lesions.

All blood and tarsal pad tissues of infected guinea pigs tested by RT-PCR and nested-PCR showed the genome of Indiana 3–Alagoas virus, as shown in Figure 2.
Figure 1: A - Guinea pig (*Cavia porcellus*); B - Inoculation of Indiana 3-Alagoas virus into the tarsal pad of the animal; C, D and E - Vesicular and crusty lesions in the tarsal pads of a guinea pig observed 3 days after infection with Indiana 3-Alagoas virus; F - Tarsal pad of a guinea pig inoculated with tissue culture medium only (negative control).
DISCUSSION

Experimental models of Vesiculovirus infection in several animal species have been described. The mouse infection showed a viral neurotropism and the migration of the virus along the olphatory nerve reaching the central nervous system (Huneycutt et al. 1994). The VS infection in pigs showed that viral transmission occurs by the contact of uninfected animals with the epithelium of ruptured vesicles from animals previously infected (Stallknecht et al. 2001).

Indiana 3–Alagoas virus was chosen for animal infection because it is a common agent of VS and has been detected in some Brazilian regions. Guinea pigs showed clear vesicular lesions in the tarsal pads two days after Indiana 3–Alagoas virus infection, simulating the VS lesions observed in large animals. Regarding to the virulence of Indiana 3–Alagoas virus in guinea pigs, it was observed, in respect to the number and the size of vesicular lesions, that they increase after three or four passages done by intradermal inoculation into the guinea pig tarsal pad with vesicular fluids of lesions from previously infected animals. After three or more passages, the Indiana 3–Alagoas virus became better adapted to the infection in animals. The animals do not die in consequence of the viral infection and the epithelial lesions of the ruptured vesicles remain for about five days. Indiana 3–Alagoas virus genome was clearly detected in the tarsal pad epithelium of the animals. Viremia was also detected in all the clinical samples showing that the infection generates a systemic disease in the infected guinea pigs.

Based on the data obtained in guinea pigs, the described RT-nested-PCR technique could be used for diagnosis of VS disease in large animals.
REFERENCES


