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ISOLATION OF AN OPHIDIAN PARAMYXOVIRUS (OPMV) IN A CAPTIVE RATTLESNAKE (*Crotalus durissus terrificus*) FROM BOTUCATU, SÃO PAULO STATE, BRAZIL

M. F. NOGUEIRA[1], T. H. BARRELLA[2], R. J. DA SILVA[3], C. A. M. LOPES[1,2], J. P. ARAÚJO JÚNIOR[1]

1 Departamento de Microbiologia e Imunologia, Instituto de Biociências, UNESP, Botucatu, São Paulo, Brasil; 2 Centro de Estudos de Venenos e Animais Peçonhentos da UNESP (CEVAP/UNESP), Botucatu, São Paulo, Brasil; 3 Departamento de Parasitologia Instituto de Biociências, UNESP, Botucatu, São Paulo, Brasil.

ABSTRACT: This study reports the isolation of an Ophidian Paramyxovirus (OPMV) in sputum of a captive rattlesnake (*Crotalus durissus terrificus*) kept in a serpentarium located in Botucatu, São Paulo State, Brazil. Polymerase chain reaction (PCR) and nested-PCR were performed for the identification of the isolated virus.
KEY WORDS: *Crotalus durissus terrificus*, rattlesnake, Paramyxovirus, Ophidian Paramyxovirus, hemagglutination, polymerase chain reaction.

INTRODUCTION

Captive snake breeding has become an important activity; first to obtain venom for antiserum production, and second to study venom pharmacological properties (6,14,15).

Infectious diseases are the major problems affecting captive snakes (2,10,11,12), especially paramyxovirosis, the agent being known as ophidian paramyxovirus (OPMV) (3,5,7,8,9). OPMV causes respiratory and neurological disorders in snakes, resulting in up to 100% mortality (4,13).

This study reports the isolation of an OPMV from the sputum of a captive rattlesnake (*Crotalus durissus terrificus*) with signs of pneumonia. The snake was kept in a serpentarium located in Botucatu, São Paulo State, Brazil.

The sputum sample was inoculated in a monolayer of Vero cells and incubated under agitation for 6 days at 30°C. A second passage was made under the same conditions. The pure secretion and cell culture supernatants were submitted to hemagglutination tests with positive results.

Polymerase chain reaction (PCR) and nested-PCR were performed with cell culture samples for the identification of the isolated virus, using the technique described by Ahne *et al.* (1) with modifications. RNA was extracted from cell culture supernatants using TRIZOL LS (Gibco BRL) as recommended by the manufacturer. cDNA was obtained by reverse transcription using Superscript RTII (Gibco BRL). In PCR and nested-PCR, the primers used were those for a partial sequence of polymerase (L) gene (1) in a reaction with a final volume of 25 μl containing: 2.5 units *Taq* DNA Polymerase (Gibco BRL); 2.5 μl PCR Buffer 10X; 10 pmols of each primer; 1.5 mM $MgCl_2$; 0.2 mM dNTP; and 5 μl of cDNA (PCR) or 2 μl of the PCR product (Nested-PCR). Conditions for amplification are shown in Table 1.

Table 1. PCR and Nested-PCR conditions for amplification of polymerase (L) gene partial sequence.

Steps	Temperature (°C)	Duration	N° of cycles
1	94	3'	1
2	94	1'	9
3	52 (-1°/cycle)	1'	9
4	72	1'30"	9
5	94	1'	25
6	44	1'	25
7	72	1'30"	25
8	72	4'	1

The positive results from hemagglutination, PCR, and nested-PCR (Figure 1) are effective to confirm that this virus is a Paramyxovirus. This is the first report about Ophidian Paramyxovirus (OPMV) in Brazil.

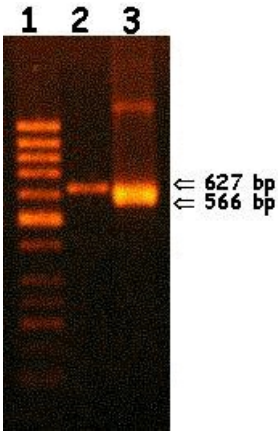

Figure 1. 627 bp (line 2) and 566 bp (line 3) bands from PCR and nested-PCR, respectively, compared with a ladder of 123 bp (line 1) of the first passage of sputum sample cell culture.

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CORRESPONDENCE TO:
M. F. NOGUEIRA - Departamento de Microbiologia e Imunologia, Instituto de Biociências, UNESP, Distrito de Rubião Júnior, CEP 18618-000, Botucatu, São Paulo, Brasil.
E-mail: furlan@ibb.unesp.br

Caixa Postal 577
18618-000 Botucatu SP Brazil
Tel. / Fax: +55 14 3814-5555 | 3814-5446 | 3811-7241
e-Mail
jvat@cevap.org.br