Two new outbreaks of *Vaccinia virus* (VACV) were observed in Midwest region of São Paulo State, Brazil. The disease occurred in one farm localized approximately 70 km from the regions previously reported one year after its first detection. In this farm the disease affected ten of sixty producer cows and 3 milkers. Subsequently, another outbreak was diagnosed two years after the first one in the same region where the disease was first observed in the Central West of São Paulo State. The lesions in affected cows were clinically less severe than the routinely observed but one milker presented a severe lesion on the finger.

The affected farms have in common the presence of rodents and eventually wild animals. Birds of different species were observed in all properties including chickens and ducks. Similar to the other outbreaks observed the animals, get started to present lesions, suggestive of VACV infection, in mammary gland and teats, that quickly disseminated between the herd. Milkers had clinical signals that included headache, limphadenopathy, fever and ulcerated lesions on fingers and fists. Material as blood and crusts were collected for laboratory diagnosis. Affected workers were sent to municipal health service for special care and blood and vesicular liquid were collected.

Extraction of genomic material of crusts and vesicular liquid were done using Invitekâ comercial kit especific for DNA. Polimerase chain reaction using hemagglutinin especific primers (EACP I and EACP II) of crusts showed amplification signal. Seven samples of crusts collected from both farms were tested resulting five positive samples. Product was sent to genome sequencing and subsequent comparison with VACV isolates already identified in outbreaks in Brazil. Sera from humans and cows were submitted to virus neutralization and ELISA test for IgG and IgM.

Thirteen among eighteen sera from cows resulted positive in virus neutralization test and IgG - ELISA test. Human sera was negative in neutralization test but positive in IgM - ELISA test indicating an acute infection process.

The occurrence of outbreaks in new areas, where the disease was never diagnosed, as well as in the region where the disease was previously observed indicates viral spread and persistence. The results again suggest that the VACV is able to persist in a reservoir not yet defined in nature. This reservoir may be responsible for viral persistence as well as viral spread and transmission.