ANIMAL INFECTIONS BY VACCINIA-LIKE VIRUSES IN THE STATE OF RIO DE JANEIRO: 1- NORTHWESTERN REGION.

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ABSTRACT

Orthopoxvirus infections in dairy cattle have been followed in the northwestern region of the state of Rio de Janeiro for the last nine years. Clinical specimens from 50 animals collected by Animal Health authorities have been received in our laboratory for laboratory diagnosis, most of them blood specimens. Orthopoxvirus infections have been confirmed in 46 animals by at least one of the laboratory tests employed: neutralizing antibody detection using as antigen a vaccinia-like strain molecularly characterized and isolated in the area; RT-PCR test; electron microscopy observation; and virus isolation in a Vero cell line. The virus strains obtained were identified as vaccinia-like by electron microscopy and reverse transcriptase polymerase chain reaction (RT-PCR), confirming the circulation of these strains in nature. Human cases associated with the infected animals have been also observed in the region, characterizing an expanding zoonosis caused by vaccinia-like strains in the state of Rio de Janeiro.

INTRODUCTION

The Poxviridae virus family includes a group of viruses which infects man, many vertebrate species, and insects. In vertebrates these viruses cause mainly but not exclusively, vesicular/pustular infections of different degrees of severity (Schatzmayr & Costa 2005). Some of the human pathogenic poxvirus infections are zoonoses. Orthopoxvirus is the most important genus in relation to human infections, including smallpox virus, eradicated as a human disease in 1977, and vaccinia virus with different strains which were used for vaccine preparation, in order to protect against smallpox.
Starting in 1999, orthopoxvirus infections have been observed in the state of Rio de Janeiro (Damaso et al. 2000, Schatzmayr et al. 2000) in dairy cattle and humans in close contact with them. The first infections were detected in the northwestern region of the state, and the studies in the area through the years since then are reported in this paper. Orthopoxvirus strains isolated in the state were characterized molecularly as vaccine-like viruses similar to the vaccinia/IOC strain, which was used in the past for vaccine preparation (Damaso et al. 2000). Other orthopoxvirus strains identified in the southeastern region of the country (Fonseca et al. 1998, de Souza et al. 2003, Nagasse-Sugahara et al. 2004, Lobato et al. 2005, Schatzmayr et al. 2005, Donatele et al. 2007) were also confirmed as vaccinia-like poxviruses, indicating that these strains are present in large areas of the country.

This paper describes studies starting in 1999 carried out in six counties of the northwestern region of the state of Rio de Janeiro, in order to confirm the presence of poxvirus in animal cases of vesicular disease and the circulation of poxvirus in nature.

Similar results have been observed in the Paraíba river valley by our group (Costa et al. 2007).

MATERIALS AND METHODS

Specimens.

Vesicular and pustular fluids, crusts and blood samples from dairy cattle showing signs of vesicular disease were received in the laboratory for diagnosis purposes. The specimens from 50 animals came from the municipalities of Cantagalo, Cordeiro, Aperibé, Santo Antonio de Pádua, Cambuci and Miracema during eight years. Serum samples were obtained from most cases and skin specimens were collected only
in acute cases, under strict ethical rules, avoiding animal pain. A total of 45 sera, 15 crusts and 2 vesicular fluids were obtained.

The cases were investigated between 1999 and 2007. Data on the cases were obtained from the owners of the animals and during the epidemiological investigations carried out on some of the farms.

**Virus isolation.**

The specimens collected from the skin lesions were treated with Eagle tissue culture medium plus antibiotics to control bacterial contamination and ground up when necessary. The material was inoculated onto a Vero cell strain sensitive to poxviruses. The inoculated cells were observed for cytopathic effects, and the presence of virus confirmed by electron microscopy and/or RT-PCR.

**Electron microscopy.**

Fragments of skin specimens were prepared for transmission electron microscopy by dilution in a small amount of distilled water and negative contrasting using PTA 1% (Brenner & Horne 1959). Observations were made at 30,000x magnification, with a Zeiss apparatus model EM-900, looking for the typical morphology of orthopoxviruses. Tissue culture fluids were contrasted and observed in the same way.
RT-PCR reaction.

Briefly, primers as described previously (Damaso et al. 2000) were applied for amplification of a segment of 1171 bp of the HA gene. The Cantagalo strain (confirmed as a vaccine-like virus) and the vaccinia virus strain Wyeth, were included as controls.

Antibody determination.

The sera were subjected to a 50% plaque-reduction neutralization test, using the Cantagalo/IOC strain as the antigen. Serum dilutions and a virus suspension containing about 40 plaque-forming units in 100 µl were incubated at 37°C for one hour in 90-well cell culture microplates, and Vero cells were added to the wells. After 48 hours, the cells were stained using crystal violet and formaldehyde 1%, washed and the plaques counted under the microscope. Tissue culture tubes were occasionally used for the test.

RESULTS

The specimens collected in the above mentioned six municipalities confirmed that orthopoxvirus strains were the etiologic agents of the vesicular disease in 46 bovines, by at least one of the techniques applied. A total of seven orthopoxvirus strains were detected by RT-PCR and six strains have been isolated.

Comparison between the methods applied to the skin specimens available showed (Table 1) that RT-PCR was the most sensitive test for the diagnosis. Electron microscopy also showed good sensitivity, all studied cases being positive for orthopoxvirus particles.
Table 1. Laboratory results of the specimens from bovines suspected of poxvirus infection in counties of the northwestern region of the state of Rio de Janeiro: 1999-2007.

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive samples</th>
<th>Negative samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT-PCR test</td>
<td>07</td>
<td>05</td>
</tr>
<tr>
<td>Virus isolation</td>
<td>06</td>
<td>05</td>
</tr>
<tr>
<td>Electron microscopy</td>
<td>06</td>
<td>0</td>
</tr>
<tr>
<td>Neutralization test</td>
<td>35</td>
<td>10</td>
</tr>
</tbody>
</table>

Antibody determinations alone were responsible for the diagnosis of most of the cases. Dilutions >1/10 were accepted as positive, although titers as high as 1/1280 have been observed. The blood collections were carried out at different stages of the disease in the animals.

Most of the cases occur during the months May to August, and according to residents similar cases of vesicular disease in cattle have been observed in the area since the smallpox mass vaccination campaign in the region, in the late 60’s. However, only after 1999 have the cases been confirmed in the laboratory.

The lesions in the adult animals were observed on the udder and teats. No lesions were detected in calves but in other parts of the state, vesicles in calves around the mouth and nose have been described (Costa et al. 2007). Vesicles/pustules reach about 2 cm in diameter and later the recovering membrane dries and falls off. The lesions then appear as bloody painful wounds, in large areas of the teat (Figure 1). The evolution of the vesicle to complete healing took 3-4 weeks and bacterial infections were observed in some cases.
Four human cases were observed in workers in direct contact with animals with lesions. Most patients only had lesions on the hands and fingers, but in one patient lesions on the face were recorded. Two of the human cases which were followed by the Health Center in Cordeiro, had been hospitalized for three days.

In the human patients, pain in the lesions, fever, ganglion inflammation, headache and prostration were described. The clinical evolution was about 3 weeks and the incubation period, after onset of the symptoms, was about one week.

**DISCUSSION**

Infections caused by orthopoxviruses in humans and animals have been described before in the state (Silva & Moraes 1961, Mesquita & Schatzmayr 1969, Schatzmayr et al. 2000). This paper describes cases observed in six counties of northwestern region of the state, where animal cases have been studied by our group since 1999.

In the region many of the farms involved in milk production have limited numbers of animals and less than optimal hygienic conditions. All cases have been
observed in farms with animals handled by milkers and not by mechanical devices for milk collection.

Smallpox vaccination in Brazil was carried out in the rural areas on a farm-by-farm system, and careless handling of the smallpox live vaccine with virus titers as high as $10^8$ / ml was usual.

These procedures most probably, allowed the dissemination of the vaccinia virus in nature, with more than one introduction. Smallpox vaccination was discontinued in the country in the 70s, but our studies confirm that vaccinia-like viruses are circulating in nature, sustaining and generating new human and animal infections. A recent review emphasized the capacity of orthopoxviruses to adapt themselves to new animal species and also confirm the presence of vaccinia-like cases in Brazil (Regnery 2007). These data demonstrate that more field studies are needed to confirm the circulation of these viruses in reservoirs like wild rodents and vectors which might be related to poxvirus transmission in the state.

Preliminary studies with wild rodents should be enlarged in the next dry season and the presence of new clinical cases is being followed in the region. One rodent collected in the area (Akodon sp.) showed neutralizing antibodies in low titer for orthopoxvirus, but more studies should be done in order to confirm the presence of rodent infections.

Other agents such as parapoxviruses, which could be demonstrated by electron microscopy techniques, have not been detected in the cases studied, although this group of viruses does circulate in the state (Barth et al. 2005).

Orthopoxvirus infections should be already considered an expanding disease of zoonotic character, causing temporarily incapacitating human disease and important
economic losses due to reduction in milk production and permanent lesions in the animals, like teats losses.

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