ANIMAL INFECTIONS BY VACCINIA-LIKE VIRUSES IN THE STATE OF RIO DE JANEIRO: 2 - PARAIBA RIVER VALLEY.

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ABSTRACT

Animal infections caused by vaccinia-like viruses strains in the Paraíba river valley, in the state of Rio de Janeiro, municipalities of Valença, Barra do Piraí, Rio das Flores, Piraí, Rio Claro e Resende, have been studied by our group. Human infections associated with the infected animals have been also observed in the area. Specimens of 102 animals were collected in 34 farms in the period May 2006 to October 2007, mainly serum samples and also crusts and vesicular fluids. The clinical specimens were submitted to a plaque reduction neutralization test, reverse transcriptase polymerase chain reaction (RT-PCR) test, electron microscopy examination and virus isolation in a Vero cell line. Orthopoxvirus infection could be demonstrated by at least one of the laboratory methods in 92 animals, and lesions were observed on the udder of dairy cattle and around mouth and nose of calves in contact with infected adults. The orthopoxvirus infections are spreading in the area and may be already considered a important zoonosis, with substantial economic losses in the dairy cattle due to reduction of milk production and permanent lesions in the udder, like losses of teats.

INTRODUCTION

The virus family Poxviridae infects man, many vertebrate species and also insects. In vertebrates these viruses cause vesicular/pustular infections of different degrees of severity (Schatzmayr & Costa 2005). Most of the human pathogenic poxvirus infections are zoonoses, with the exceptions of smallpox virus and molluscum contagiosum. Orthopoxvirus is the most important genus in relation to human infections, including smallpox virus, eradicated as a human disease in 1977; vaccinia virus with different strains,
used for vaccine preparation; cowpox, a zoonosis occurring in Europe and parts of Asia and probably the ancestral virus of the group, and monkeypox, which circulates in wild animals and infects man, mainly in Africa; this virus was recently introduced into North America by the importation of African rodents for sale as pets, and spread to prairie dogs (*Cynomis* sp.) (CDC 2003).

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. Poxvirus strains isolated in the state over the years were characterized 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 isolated in the southeastern region of the country (De Souza et al. 2003, Nagasse-Sugahara et al. 2004, Lobato et al. 2005) were confirmed as vaccinia-like poxviruses. The last-named paper describes a human outbreak of vaccinia-like virus in counties along the Paraíba river valley in the state of São Paulo, showing that in that region poxviruses are important disease agents.

This paper describes studies carried out in six counties of the Paraíba river valley in the state of Rio de Janeiro during one and a half year, aiming to confirm the presence of poxvirus in vesicular infections in animals and humans, and to contribute towards a better understanding of the circulation of poxviruses in the state. Infections by poxviruses have also been studied by our group in the northwestern region of Rio de Janeiro state (Simonetti et al 2007).
MATERIALS AND METHODS

Specimens.

Vesicular and pustular fluids, crusts and blood samples were received in the laboratory for diagnostic purposes from dairy cattle showing signs of vesicular disease. The specimens came from the municipalities of Valença, Barra do Piraí, Rio das Flores, Pirai, Rio Claro and Resende in the Paraíba river region of the state of Rio de Janeiro. The specimens from 102 animals were sent to the laboratory by animal health authorities. 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 90 sera, 20 crusts and 2 vesicular fluids were collected.

The cases had been investigated between May 2006 and October 2007, in 34 farms distributed in the afore-mentioned counties. Data on the cases were obtained from the owners of the animals and during the epidemiological investigation carried out by one of the authors (RVVC).

Virus isolation.

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

Antibody determination.

The sera were submitted to a 50% plaque-reduction neutralization test, using the Cantagalo/IOC strain as 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 cell culture microplates and Vero cells were added to the plates. After 48 hours, the cells were stained using crystal violet and formaldehyde 1%, washed and the virus plaques counted under the microscope. Tissue culture tubes were occasionally used for the neutralization tests.

RESULTS

The specimens collected in the above mentioned six municipalities confirmed that orthopoxvirus strains were the etiologic agents of the vesicular disease in 92 bovines, by at
least one of the techniques applied. A total of 15 orthopoxvirus strains were detected by RT-PCR and 9 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 showed also good sensitivity, in comparison with the virus isolation method.

Table 1. Laboratory results of the clinical specimens from bovines suspected of poxvirus infection.

<table>
<thead>
<tr>
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<th>Positive samples</th>
<th>Negative samples</th>
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<tr>
<td>RT-PCR test</td>
<td>15</td>
<td>04</td>
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<tr>
<td>Virus isolation</td>
<td>07</td>
<td>09</td>
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<tr>
<td>Electron microscopy</td>
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<td>09</td>
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<tr>
<td>Neutralization test</td>
<td>65</td>
<td>20</td>
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Antibody determinations alone were responsible for diagnosing most of the cases. Serum dilutions >1/10 were accepted as positive, but the great majority of the positive sera showed higher titers, up to 1/1280.

The county of Valença represents 35.5 % of the farms with cases, followed by Barra do Piraí with 19.3% and Resende with 16.1%.

Most of the cases occurred during the months May to August; only 4 out of 34 farms had cases in other periods. According to information obtained from local residents, similar episodes of vesicular disease have been observed during recent years and even just after the smallpox mass vaccination campaign, about forty years ago.

The attack rate, calculated from the adult dairy cattle infected and not infected in each farm, was higher than 60% in 52 % of the farms and in 32% of the farms the attack
rate reached less than 60%. The disease was transmitted only to calves drinking from infected mothers but not to the calves drinking milk from containers, suggesting the absence of virus in the milk of the infected animals.

The lesions in the adult animals were observed on the udder and teats (Figure 1 A, B, C) and in the lips and around the noses of calves (Figure 1 E, F), seldom in the gums and tongue (Figure D). Typically after the vesicle stage (1-2 cm diameter), the recovering membrane dried and the lesion changes to a bloody painful wound, sometimes covering large areas of the teat. The evolution of the vesicle to the complete healing took 3-4 weeks.

Ten human cases were observed in workers in direct contact with animals with lesions, and no human to human transmission could be confirmed. Most patients only had lesions on the hands and fingers, but in four patients lesions on the face were also recorded. Almost all patients could not perform their normal activities for at least one week during their illness.

In the human patients pain in the lesions, fever, regional ganglion inflammation, headache and prostration were described. The clinical evolution lasted about 3 weeks and the incubation period after the first contact with the infected animals was of 5 to 7 days.

**DISCUSSION**

Infections caused by orthopoxviruses in human and animals have been described in different countries (Lum et al. 1967, Topciu et al. 1976, Schatzmayr et al. 2000). An outbreak of vaccinia virus infections in the same area during the smallpox mass vaccination campaign, with human and animal cases, has been described (Mesquita & Schatzmayr 1969).
Figure 1. Lesions in the udder of adult animals and in tongue and around the noses of calves (arrows).
Nagasse-Sugahara et al. (2004), studying 74 cases of a vesicular human disease in the Paraíba river valley in the state of São Paulo, indicated that the valley is an area of circulation of poxviruses, which has been confirmed in this paper.

The smallpox vaccination in Brazil, was carried out in the rural areas, farm-by-farm, and careless handling of a live vaccine with high virus titers most probably allowed the dissemination of the vaccinia virus in nature. It is likely that vaccine strains had been introduced into nature more than once.

Other authors have confirmed through the years the presence of animal and human infections with poxvirus, especially in the southeast region (Mello et al. 1960, Silva & Moraes 1961, De Souza et al. 2003, Schatzmayr et al. 2005, Donatele et al. 2007).

It should be remembered that smallpox vaccination was discontinued in the country in the 70s, and this paper confirms that vaccinia-like viruses are circulating in the country, a fact already accepted in the international literature (Regnery 2007).

The vesicles occurred on the udder, particularly on the teats, with confluence of vesicles resulting in large lesions covered by crusts. Oral lesions were observed in three calves, one of them with lesions around the nose and on the tongue. Both the PCR test and serology showed positive results in this animal. On one of the farms, animals presenting vesicular/pustular lesions, were negative by all laboratory techniques applied. Other agents were probably involved in those cases, perhaps parapoxviruses.

Permanent udder lesions were observed as well as mastitis, which resulted in economic losses.
Our PCR results showed that the strains obtained in animals matched the Cantagalo strain, reconfirming that vaccinia-like virus strains circulating in the Paraíba valley are closely related to the original Cantagalo strain, which was described as originating from one of the strains used for vaccine preparation at the Oswaldo Cruz Institute (Damaso et al. 2000).

In the six municipalities studied the disease was confirmed in the laboratory. Those municipalities belong to the Paraíba river area, but the presence of vesicular/pustular infections in other counties like Vassouras and Três Rios has also been described but not yet confirmed in the laboratory. A surveillance system has been established and other municipalities are being monitored.

A recent review (Regnery 2007) emphasized the capacity of orthopoxviruses to adapt themselves to new animal species. These data confirm that field studies are needed for confirming the possible role of reservoirs like wild rodents and vectors which might be related to poxviruses transmission in the state.

According to the data obtained over recent years, orthopoxvirus infections are spreading in the state of Rio de Janeiro, and should be already considered an emerging disease of zoonotic character, causing temporarily incapacitating human disease, which may be more severe in individuals with low cellular immunity. In dairy cattle, such infections may cause important economic losses due to reduction in milk production and permanent lesions on the udder.
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