

Research Article

ANTIBODIES ANTI-ORTHOPOXVIRUS IN HEALTHY WORKERS WHO HANDLE ANIMALS AND IN PATIENTS WITH CUTANEOUS LESIONS COMPATIBLE WITH POXVIRUS INFECTION IN THE STATE OF RIO DE JANEIRO, BRAZIL

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ABSTRACT

The incidence of poxvirose, a zoonosis caused by viruses of the family *Poxviridae*, has increased over the past two decades in Brazil. The important features of the Orthopoxvirus infection include rapid spread, occurrence restricted to lactating cows, lack of hygiene, person-to-person spread, and occurrence at properties where milking is conducted. We detected the presence of vaccinia virus antibodies, specifically for Cantagalo virus, in samples from two distinct populations from the state of Rio de Janeiro: (i) healthy professionals who live in the State of Rio de Janeiro and handle animals and (ii) in patients with cutaneous lesions compatible with poxvirose. The plaque reduction neutralisation test (PRNT) was used. Of the 136 samples from the group of healthy professionals, 12 displayed seroreactivity with titres lower than or equal to 1/10. Anti-poxvirus antibodies were detected in 68.3% of samples of patients suspected to be infected with poxvirus (28/41). Therefore, the plaque reduction neutralisation test constitutes a sensitive assay that can be more commonly used for diagnosis. Furthermore, professionals who handle animals should be included as an occupational risk group in areas with confirmed human poxvirus cases.

Keywords: Orthopoxvirus, antibodies, healthy workers, patients, Rio de Janeiro State, Brazil
II Simpósio Avançado de Virologia Hermann Schatzmayr

**** Note from authors:** This article is dedicated to the memory of Dr. Hermann Schatzmayr, who was one of the most important virologists from Brazil. A national and international reference on virology, as well as a participant in efforts to eradicate smallpox and poliomyelitis, he was a pioneer in the study of myxoviruses, poxviruses, viral gastroenteritis, and viral hepatitis, among other viruses, and was responsible for the isolation of dengue virus strains 1, 2, and 3 in Brazil. We will always be grateful for his teaching, kind encouragement and generosity.

INTRODUCTION

Poxvirus infections involving cattle and humans, primarily persons linked to rural areas, have been described in different countries (Lum et al. 1967, Topciu et al. 1976) and in Brazil (Mesquita & Schatzmayr 1969, Schatzmayr et al. 2000). The incidence of poxvirose, a zoonosis caused by viruses of the family *Poxviridae*, has increased over the past two decades in Brazil, mainly in the southeast region of the country. Schatzmayr et al. (2000) described an outbreak of infections in dairy cows and humans on a farm in the municipality of Cantagalo in the mountainous region of the State of Rio de Janeiro after the end of the national campaign to eradicate smallpox.

Damaso et al. (2000) have shown that the virus, called Cantagalo (CTGV), isolated in municipalities

with animals exhibiting vesicular/pustular disease is an Orthopoxvirus and that at the molecular level, this virus is very close to the sample vaccinia virus used in the past to prepare vaccines at the Instituto Oswaldo Cruz. Later, other authors confirmed the circulation of similar viruses as a cause of infection in animals and humans, especially in the state of Rio de Janeiro and throughout southeastern Brazil (Costa et al. 2007, Donatele et al. 2007, Leite et al. 2005, Lobato et al. 2005, Nagasse-Sugahara et al. 2004, Schatzmayr et al. 2009a, Schatzmayr et al. 2009b, Schatzmayr et al. 2011, Simonetti et al. 2007, Trindade et al. 2003, Trindade et al. 2006, Trindade et al. 2007). The movement of vaccinia virus has been confirmed in the Brazilian territory and by reports in the international literature (Regnery 2007).

The important features of the orthopoxvirus infection include rapid spread, occurrence restricted to lactating cows, poor hygiene and occurrence of almost

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all of the cases at properties where milking is conducted. Person-to-person spread in a family environment from direct contact has been observed in some reports of infections caused by poxvirus (Damaso et al. 2000, Lobato et al. 2005, Schatzmayr et al. 2011, Trindade et al. 2006).

In the human population, the time that elapses between the first contact with an infected cow's udder during milking and the appearance of the first lesions in the hands of a milker, the incubation period, is on average a week, followed by an evolution of the disease from 15 to 30 days. Some patients experience severe symptoms and require hospitalisation. The seasonal nature of the disease, with a prevalence of cases in the dry season, has been identified in different studies (Damaso et al. 2000, Donatele et al. 2007, Leite et al. 2005, Lobato et al. 2005, Simonetti et al. 2007).

Whereas poxvirosis is considered an emerging viral disease associated with occupational exposure, this study aims, in addition to providing more complete information on the movement of poxviruses in the territory of the state, to check for the presence of antibodies against vaccinia virus, specifically Cantagalo virus, in samples from two distinct populations in the state of Rio de Janeiro: (i) healthy professionals who have handled animals and lived in the State of Rio de Janeiro, and (ii) patients with cutaneous lesions compatible with poxvirosis.

MATERIAL AND METHODS

Study population

Serological tests for the presence of antibodies against Orthopoxvirus were conducted in two distinct human populations from the state of Rio de Janeiro. The first studied population was composed of 136 healthy professionals, residents of the State of Rio de Janeiro, from different educational and research institutions who handled domestic and wild animals, such as veterinarians, researchers, mastozoologists, biologists, and taxonomists, during the period from 2008 to 2009. The second population consisted of 41 patients from eight counties in the state of Rio de Janeiro with notification of suspected cases of poxvirus infection during the period from 1999 to 2011. The patient samples were sent in the context of epidemiological surveillance to the Laboratory of Morphology and Viral Morphogenesis (LMMV), Instituto Oswaldo Cruz, Fiocruz, and kept at -20 °C.

Poxviruses are not reportable diseases except for smallpox. Clinical samples of patients suspected of poxvirosis were sent, by spontaneous demand and without a detailed epidemiological form, to the LMMV. For the cross-sectional study with healthy professionals who handle animals, all participants, after signing the consent form (TCLE) and completing a questionnaire prepared specifically for this study, underwent collection of 5-10 mL of blood for research, including study of the

zoonotic agent of poxvirus.

Laboratory tests

The plaque reduction neutralisation test (PRNT) can detect antibodies specific to poxvirosis and is not specific to the Cantagalo virus. The sera, previously diluted 1/10 in Eagle-Earle culture medium and inactivated at 56°C in a water bath, were applied at a volume of 50 mL to each well of a microplate for tissue culture cells and diluted successively with 50 µL of culture medium from the initial 1/10 dilution to 1/1280. Subsequently, a Cantagalo virus sample, containing from 30 to 60 plaque-forming units, was added to the plate. After incubating the plate at 37 °C for one hour, a suspension of Vero cells in culture medium containing foetal bovine serum was added. Then, after incubation at 37 °C for 48 hours, the cells were stained with a solution of 1% crystal violet diluted in 20% formalin for 30 minutes. The dye was removed from the plate with careful washing to not damage the cells. The plate was again placed in an oven at 37 °C for drying until assessment of the cell layer, and counting of the plaques was performed using a light microscope. The maximum dilution that resulted in a 50% or more reduction in the number of plaques compared with the virus control was considered the serum neutralising titre (modified from Herring et al. 1990).

Ethical considerations

The study of samples of suspected cases of poxvirosis and the population of healthy professionals who handle animals were reviewed and approved by the Ethics in Research Fiocruz under the registration numbers 486/08 and 409/07, respectively.

RESULTS

During the study period, 177 samples were analysed of which there were 136 professionals who handle animals living in the municipality of Rio de Janeiro and 41 samples of suspected poxvirosis in human cases from different municipalities of the State of Rio de Janeiro.

Detection of antibodies by plaque reduction neutralisation test (PRNT) in the healthy professional group.

Of the 136 samples of the healthy professional group, 12 professionals displayed seroreactivity with titres higher than or equal to 1/10. Ten of the seroreactive professionals were female, and two were male. Except for three students (ages 21, 28 and 33 years), all were older than 40 years of age.

Detection of antibodies by plaque reduction neutralisation test (PRNT) in the group of patients with clinical signs compatible with poxvirosis.

Anti-poxvirus antibodies with titres higher than or equal to 1/10 were detected in 68.3% of human samples analysed (28/41). Seroreactivity was identified

in 11 samples from municipality of Barra Mansa. The antibody titers changed from 1/20 (five samples) to 1/40 (three samples, 1/80, 1/160 and 1/320 (one sample of each)). Proceeding from the Valença municipality, the samples analyzed showed antibody titers changing from 1/20 and 1/40 (two samples of each) to 1/80 (one sample) and 1/160 (two samples). The six samples from the Rio Claro municipality showed titers of 1/20 (five samples) and 1/80 (one sample). The two samples from Itaocara municipality showed a titer of 1/20. One sample from each municipality of Cordeiro and Parati presented 1/40. The antibody titer was below 1/10 of the three samples and one sample from Pirai and Miracema municipalities, respectively.

There was a general lack of an epidemiological form to provide clinical and epidemiological data concerning the patients suspected of poxvirolosis. The patient's age was recovered for only 11 from 17 samples of the Barra Mansa municipality. Three of them presented ages between 21 and 30 years, two between 31 and 40 years and six were elder than 41 years.

DISCUSSION

Orthopoxvirus infections in humans and animals have been described in different countries (Lum et al. 1967, Mesquita & Schatzmayr 1969, Topciu et al. 1976). In Brazil, since the 1990s, the identification and diagnosis of cases have increased, in particular in the Southeast region, as has the interest in the study of this underestimated and underreported zoonosis.

Starting with the analysis of serum samples from a group consisting of patients with suspected poxvirolosis lesions, it was possible to identify serologic evidence of poxvirus infection in six of the eight municipalities investigated. An analysis of demographic, clinical and epidemiological data was not possible as only 22 of the 41 standard poxvirus surveillance questionnaires sent by the health services provided data such as patient age or smallpox vaccination status. Of these 22 cases with more detailed demographic information, 18 presented positive samples and eight had serological titres higher than or equal to 80. Of these cases, six patients were older than 40 years, an age group that certainly was subjected to the vaccination campaigns against smallpox.

Although the number of patients is small, and it has not been possible to recover information about previous vaccinations, these results lead to an investigation of patients older than 40 years and previously vaccinated on the hypothesis of a partial and not permanent immune response because the observed titres could be the result of a "boost" of the immune system. Thus, although the study design did not allow any conclusion on the subject and the results may be due to the vaccination campaigns against smallpox, the real possibility that some of the seroreactive individuals have acquired infection from contact with animals must be

considered. This assumption is reinforced by the group's experience in the field, where it was observed that the majority of the male milkers younger than 40 years were seronegative.

Vaccination against smallpox was conducted in Brazil in urban areas from house to house in rural areas and from farm to farm in agricultural areas (Schatzmayr 2001). The lack of experience in handling the attenuated vaccine with high titres (approximately 108 virus particles per mL) most likely allowed the introduction and spread of strains of vaccinia virus in nature. It is likely that the vaccine strains have been introduced into nature more than once. Over the years, several authors have confirmed the presence of infections in animals and humans by poxvirus, particularly in the Brazilian southeast (Mello et al. 1960, Schatzmayr et al. 2009a, 2009b, 2011); Silva & Moraes 1962, Trindade et al. 2003, Donatele et al. 2007). It must be remembered that the smallpox vaccination campaign was discontinued in the country in the 1970s, which confirms that the vaccinia-like virus is circulating in the country, a fact accepted in the international literature (Regnery 2007).

Nagasse-Sugahara and colleagues (2004) studied 41 cases of vesicular disease in humans in the Paraíba do Sul river valley, state of São Paulo, Brazil. They concluded that this region is the preferred area of movement of poxviruses, which was confirmed by this and other studies (Costa et al. 2007, Damaso et al. 2000, Lobato et al. 2005, Trindade et al. 2006).

The group of healthy professionals consisted of 136 samples collected from different healthy professionals who had direct contact with different types of animals and who did not display any suggestive clinical poxvirolosis. All of these professionals were residents in the metropolitan area of the municipality of Rio de Janeiro. Starting with the data registered on a structured questionnaire, it was verified that of the 12 samples that reacted with titres above 1/10, nine were professionals older than 40 years. Ten were women, and only two were male. Three seropositive samples were from students younger than 30 years of age. As antibodies to poxvirus from these professionals have been observed, this suggests the need to include people who handle animals, especially wild animals, as an occupational risk group. In this context, considering the lack of information on the subject in the scientific literature, it is recommended that surveillance be conducted not only in milkers but also in professionals with close ties to animal handling.

Some case reports have indicated total ignorance regarding the complexity of the epidemiology of this zoonosis. For example, concerning the identification of viral transmission between humans, as reported by Batista et al. (2009) and by Schatzmayr et al. (2009), cases processed in the Laboratory of Morphology and Virus Morphogenesis, Instituto Oswaldo Cruz, Fiocruz, include a description of a vulva infection through direct

intra-family contact with lesions on the husband's hands. This was the first time such a case was described in Brazil.

Therefore, the plaque reduction neutralisation test constitutes a sensitive assay and is the most commonly used technique for diagnosis. It may become difficult to implement this technique due to the required availability of excellent cell culture reagents, lack of contamination and susceptibility to replication of a high titre of virus and experienced professionals for examining the cell culture plates.

Although considered an infection that affects rural workers, given the complexity of the maintenance cycle of poxviruses in nature, it is strongly recommended that professionals who handle animals, especially ruminants and wild animals, to be included as an occupational risk group in areas with confirmed human cases.

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