Letter to the Editor

Presence of neutralizing antibodies to *Orthopoxvirus* in Capybaras (*Hydrochoerus hydrochaeris*) in Brazil

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Abstract

Cantagalo virus is a strain of vaccinia virus (genus *Orthopoxvirus*) and the etiological agent of an important vesicopustular disease that affects dairy cows and milkers in Brazil. The reservoirs involved in the maintenance of this virus in nature are unknown. In the present work, the detection of neutralizing antibodies to *Orthopoxvirus* in capybaras collected in São Paulo state is reported. Capybaras are the largest rodent species native to South America and have already been reported as putative reservoirs of other pathogenic microorganisms. Thirteen out of thirty-three serum samples were found positive in plaque-reduction neutralization tests, some of them showing high titers compared to positive controls. These results suggest that capybaras may play a role in the infection cycle of vaccinia virus in Brazil.

Key words: Cantagalo virus; vaccinia virus; poxvirus; capybara; *Orthopoxvirus*; animal reservoir.

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Introduction

Cantagalo virus (CTGV) is a strain of vaccinia virus (VACV; *Poxviridae* genus *Orthopoxvirus*) originally isolated in Brazil in 1999 [1]. The disease is characterized by the appearance of pustular lesions on the skin of the udder and teats of dairy cows, followed by fever, and sometimes secondary mastitis. Dairy workers acquire the disease after milking infected cows. In humans, the clinical symptoms are high fever, lymphadenopathy, headache and malaise [2]. Over the last 15 years VACV infection has spread to several states of Brazil raising economical and occupational concerns for the dairy agribusiness. Most of the etiological agents of the outbreaks in farms have been identified as VACV strains similar to CTGV (CTGV-like) [3-6]. Nevertheless, other strains of VACV distinct from CTGV have also been isolated but not recurrently [2,7].

It is widely accepted in the literature that vaccinia virus has no animal reservoir and its origin is unknown [2]. Nevertheless, the endemicity of VACV infection in Brazil challenges this assumption, suggesting the involvement of an animal reservoir in its transmission cycle. The transmission of Brazilian VACV strains has been mainly associated with the movement of dairy workers between farms, animal trade and neighboring farms [4]. Some studies have investigated the involvement of peridomestic and wild animals as putative reservoirs for VACV in Brazil without reaching definite conclusions [7,8]. Moreover, rodents are the primary targets of investigation based on studies conducted on other orthopoxviruses such as monkeypox and cowpox viruses [9,10].

During our studies on the identification of CTGV infection in dairy herds, we have regularly found livestock testing positive in which the cause of infection could not be associated to one of the three epidemiologic links cited above, *i.e.* movement of dairy workers between farms, animal trade and neighboring farms. Inquiries have also ruled out the increase of small peridomestic rodents in those farms during the outbreaks. Interestingly, the farmers usually indicated an increase in the surrounding population of capybaras (*Hydrochoerus hydrochaeris*). Capybaras are the largest rodent species in the world and are commonly found in savannas, forests and wetland areas in South America. They are regularly found in close proximity to rivers and swamps in farms and parks in Brazil. The role of these animals as asymptomatic hosts for zoonotic pathogens has not

been widely investigated, but there have been reports showing the presence of *Toxoplasma gondii*, *Trypanosoma evansi* and Rickettsia-infected ticks, as well as studies investigating the seroprevalence of *Trypanosoma cruzi*, *Leishmania infantum*, *Encephalitozoon cuniculi*, *Sarcocystis neurona*, and *Neospora caninum* in these rodents [11,12].

The Study

In this study we evaluated the presence of neutralizing antibodies against *Orthopoxvirus* in 33 serum samples of captive and wild-caught capybaras obtained from six counties of São Paulo state between 2004 and 2006 [11] (Table 1). Detection of neutralizing antibodies was evaluated by plaque-reduction neutralization test (PRNT) based on a method previously described [13]. Serial two-fold dilutions of inactivated serum samples were incubated with VACV strain Western Reserve (WR) for 1 hour at 37°C and then added to monolayers of BSC-40 cells for two hours. Cells were then washed and incubated with fresh medium for a further 40 hours at 37°C. Viral plaques were visualized and counted after fixation with formaldehyde/crystal violet. A serum sample was considered positive when it reduced the number of viral plaques by at least 50% in a 1:20 dilution. $PRNT_{50}$ titers were calculated as the reciprocal value of the serum dilution that inhibited the number of viral plaques by 50%. Serum from a laboratory worker vaccinated repeatedly with VACV [13] and serum from a dairy cow PCR-positive for CTGV infection [4] were used as positive controls showing titers of 320 and 37, respectively. Serum obtained from a dairy cow PCR-negative for CTGV [4] was used as negative control for PRNT showing titers < 5.

As shown in Table 1, 13 serum samples (39.4%) were found positive for detection of *Orthopoxvirus* neutralizing antibodies in three independent assays. Figure 1 shows a representative PRNT assay for positive and negative sera. Due to limited availability of the sera, it was not possible to reproduce the results for other 6 samples and they were therefore non-confirmed positive cases (Table 1). Two other serum samples (6%) were considered borderlines because titers were 18 and 17. It is noteworthy that five of the 13 positive serum samples (38.5%) from capybaras had high neutralization titers (> 80). These values were higher than titers found for a serum sample of a cow infected with CTGV (titer of 37) which was used as positive control.

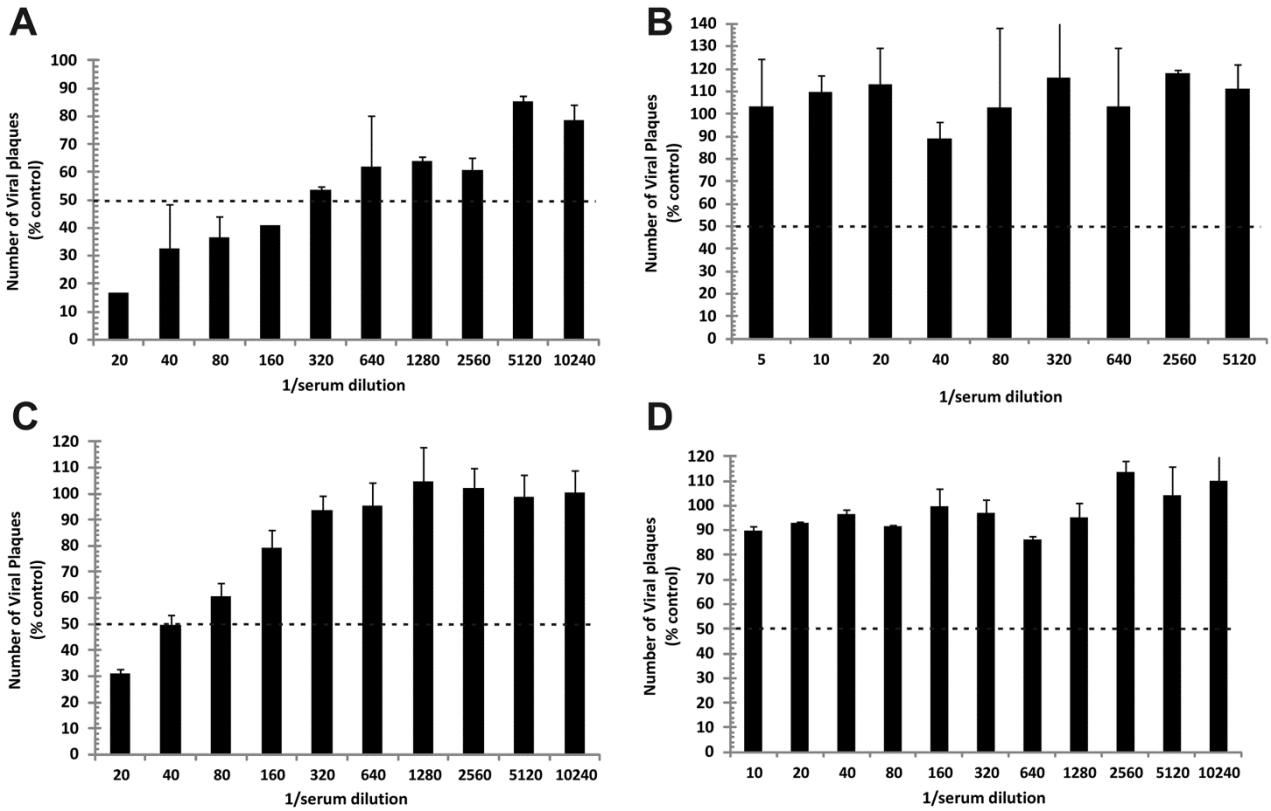
Positive serum samples were also PRNT-assayed using Myxoma virus as neutralization target instead of VACV. It is worth noting that poxviruses that belong to the same genus show serological cross-protection and cross-reactivity. Myxoma virus is a poxvirus of the *Leporipoxvirus* genus and therefore should not be cross-neutralized by anti-*Orthopoxvirus* antibodies. As expected, Myxoma virus infection was not neutralized even at 1:20 dilutions of the sera (data not shown). Therefore, neutralization antibodies detected in positive samples were specific for *Orthopoxvirus*. In addition to capybaras, we also tested 20 serum samples of another species of wild rodent, the Brazilian agouti (*Dasyprocta aguti*) collected in Santarém (2°26'35"S and 54°42'30"W), Pará state, as described in literature [14]. All samples tested negative for the presence of *Orthopoxvirus* neutralizing antibodies (data not shown).

Table 1. Number of capybaras tested positive for *Orthopoxvirus* neutralizing antibodies and $PRNT_{50}$ titers.

County/year (coordinates)	No. seropositive animals/total no. animals	No. animals per $PRNT_{50}$ titer				
		20-40	40-80	80-160	160-320	≥320
Cordeirópolis/ 2004 (22º 28' 55" S and 47º 27' 24" W)	7/8	3[a]	2	2	0	0
Cosmorama/ 2006 (20º 28' 40" S and 49º 46' 40" W)	2/2	1	0	0	1	0
São Paulo/ 2004 (23º32'51" S and 46º38'10" W)	3/5	0	2	0	1	0
Ribeirão Preto/ 2005 (21º 10' 39" S and 47º 48' 37" W)	7/13	0	1	0	1	5[c]
Valparaíso/ 2005 (21º 13' 40" S and 50º 52' 06" W)	0/3	0	0	0	0	0
Andradina/ 2005 (20º 53' 46" S and 51º 22' 46" W)	2/2	1[b]	0	0	0	1[c]
Total	**21/33**	**5**	**5**	**2**	**3**	**6**

[a, b] One sample was considered borderline with titer of 17[a] or 18[b]; [c] Samples considered suspicious (non-confirmed positive). Results obtained from one assay.

Figure 1. Detection of neutralizing antibodies against *Orthopoxvirus* in serum samples from capybaras by PRNT. Representative PRNT assays are shown. **(A)** Positive serum from an animal collected in Cordeirópolis county; **(B)** Negative serum from an animal collected in São Paulo county; **(C)** and **(D)** Positive and negative control sera collected from cows tested PCR-positive and PCR-negative for CTGV, respectively.



The results obtained in this work point toward previous exposure of capybaras to orthopoxviruses. To our knowledge, this is the first report suggesting a role of large-size rodents in the infection cycle of orthopoxviruses. In this context, VACV is the only *Orthopoxvirus* known to be the cause for infection in Brazil and CTGV-like isolates are the most prevalent strain [2]. It is worth noting that there has been no report on clinical signs of vesicular disease or other symptoms related to VACV infection in capybaras. In addition, according to the epidemiologic surveillance system SIVCONT, outbreaks of VACV infection in dairy herds have never been officially reported in the six counties of São Paulo state where data was collected [15]. Nevertheless, outbreaks of VACV infection have been repeatedly reported in São Paulo state after 1999 in different counties located 49 to 300 km away from the counties indicated in Table 1 [5,6,16]. Therefore, it will be interesting to further investigate a possible role of capybaras as reservoirs of *Orthopoxvirus* in Brazil, especially for vaccinia virus strains, such as Cantagalo virus and other similar strains that are the etiologic agents of an endemic zoonosis already widely spread in several Brazilian states. In addition, capybaras have a widespread distribution in rural and urban regions of Brazil and are also found in other South American countries, except for Chile. The identification of animal reservoirs for Brazilian strains of VACV will certainly contribute to limiting the spread of the virus to other regions of Brazil and also to neighboring countries, where these animal species also reside.

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