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Cross-Protection of Different Vaccines Against Three Divergent Wild Animal Mexican Molecular Variants of Rabies Virus

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Abstract: Now that canine rabies in Mexico has been controlled, most of human and animal cases are due to variants from wildlife: vampire bats, skunks and others. Although, most variants are similar in their genome, a skunk variant from Baja California Sur differs up to 19% in its genome from other rabies isolates. We wanted to test the efficiency of various commercial veterinary and human vaccines in protecting this and other wildlife viruses; the inactivated PV strain vaccine used in dog vaccination campaigns in Mexico; the recombinant vaccinia-rabies VRG vaccine, usually used by oral route was administered by parenteral route; the human vaccine produced in diploid cells (PM 3-1503 strain) and the human-used vaccine produced in VERO cells (PM strain). Three different isolates were used as challenges viruses: vampire bat, lynx/fox and hypervariable skunk virus. The protection proffered was tested by the NIH test. All 4 vaccines tested protected above the WHO's requirements: VRG administered intramuscularly conferred the highest protection (> 5 IU). All vaccines evaluated were efficient against the skunk isolate (>5, 4, 4 and 7 IU, respectively). PV strain vaccine conferred the least protection. All vaccines tested were efficient against the hypervariable skunk isolate and also the other wildlife strain tested.

Key words: Rabies vaccines, rabies, divergent isolates, NIH test

INTRODUCTION

Dog rabies in Mexico has been controlled by massive vaccination of this species. Now with dog rabies controlled, domestic animals are now exposed to rabies strains from other species, such as vampire bats, skunks or coyotes. In fact most human rabies cases in Mexico are now due to bat and skunk rabies variants, mostly in rural areas (74.8%) (De Mattos *et al*., 1999). In addition in August 2004 a child died at rabies transmitted by a wildcat which, in turn, had been infected by a vampire bat.

For this reason it was of interest to us to know how efficient different commercially available vaccines are in protecting against different wild animal rabies isolates.

The antigenic features from different rabies variants in Latin America have been studied (Loza-Rubio *et al*., 1996; Velasco *et al*., 2002; Favoretto *et al*., 2002; Cisterna *et al*., 2005; Nadin-Davis and Loza-Rubio., 2006). By means of molecular tools, we and others have noticed the importance at these differences in helping control rabies (Tordo *et al*., 1986; Velasco *et al*., 2006). One important fact that is usually overlooked is that the interaction of the human and domestic animals populations with wild fauna has increased. In some recent studies we sequenced a rabies variant from skunks in Baja California Sur, Aguascalientes and San Luis Potosi (Central, Mexico) and found that this variant differs in approximately 19% of its genome from other variants from vampire-bats and terrestrial mammals (Loza-Rubio *et al*., 1998; De Mattos *et al*., 1999). These genetic differences also could suggest antigenic differences. Lodmell *et al*. (1995) tested 4 vaccines against 17 rabies variants from different countries worldwide, they observed that sera from mice infected with any one virus variant cross-neutralized all of the other viruses, so they concluded that a single rabies virus strain or its G protein would protect

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globally against wild-type rabies viruses. There are other studies in which researchers have analyzed cross-protection among rabies Human Diploid Cell Vaccine (HDCV) and European and Australian bat lyssaviruses, showing a strong evidence for broad spectrum cross-neutralisation and cross protection of phylogroup I lyssaviruses using rabies HDCV (Brookes *et al*., 2005). More recently, Müller tested 2 different oral vaccines used in Germany for vaccinating foxes and raccoon dogs: SAD-B19 and SAD P/5/88 against European bat lyssaviruses type 1 and 2. However, in Mexico despite having found several variants of rabies virus, it is not wise degree of protection afforded by most commercial vaccines used in the country for human and veterinary use. Thus, in the present study we tested the efficacy of four antirabies vaccines, 2 for humans and 2 for animals, against three representative Mexican wild variants: vampire bat, lynx/fox and skunk variant mentioned.

MATERIALS AND METHODS

This study was conducted during the year 2004, at the facilities of the National Center of Veterinary Research, INIFAP.

NIH test: This test was used to determine the potency level of each of the four vaccines. The NIH procedure was followed according to Wilbur and Aubert (1996). This technique is a volumetric method for calculation of potency. The results are expressed as International Unit (IU) which represents the relative potency of the tested vaccines. As vaccine we used the reference β-propiolactone-inactivated VERO cell vaccine from the International Laboratory for Biological Standards (Denmark), containing one IU per milliliter. The WHO's recommendation for antirabies vaccine is 1 IU for animals and 2.5 IU for humans.

Vaccines: The tested vaccines were: Vaccine A: the recombinant vaccinia-rabies VRG vaccine administered usually to wild animals orally (foxes, coyotes and raccoons) (Hanlon *et al*., 1998; Grosenbaugh *et al*., 2007). Although, it was administered here by the parenteral route. Vaccine B: A nationally produced inactivated adjuvated vaccine (PV strain) extensively used in official canine rabies campaigns in Mexico. Vaccine C: Inactivated human diploid cell vaccine (Pitman Moore 3-1503-3M) (Bahmanyar *et al*., 1976; Brookes *et al*., 2005) and vaccine D: inactivated human VERO vaccine (Wistar rabies PM) (Suntharasami *et al*., 1986). All vaccines were twice applied intraperitoneally (7 days apart), in different dilutions Log_5 (1:5, 1:25, 1:125 and 1:625, volume of 0.5 mL) to 16 mice, for each dilution as indicated by the WHO technique.

As wild virus challenges: The Mxbv 3137 isolate from a bovine bitten by a vampire bat from Chiapas (Southest Mexico) (Loza-Rubio *et al*., 1999); the Mxgm 3148 isolate from a lynx captured in Chihuahua (Northwest of Mexico) and the Mxsk 3136 isolate from a skunk (divergent cycle) captured in Baja California Sur (Northwest Pacific Coast). These three challenge isolates were adjusted to 50 $MICLD_{50}$ in 0.03 mL and injected into vaccinated mice (and controls), 7 days after the last vaccination, according with the technique previously described.

The three isolates belong to the sero/genotype 1, which is the only one that has been reported in Mexico and in the whole continent (Loza-Rubio *et al*., 2006; Nadin-Davis and Loza-Rubio, 1996; Velasco *et al*., 2006).

Genomic differences among the three wild life rabies isolates: As a preamble to the immunological study, the variation among the different challenge isolates was determined by sequencing a segment between the G and L genes approximately (600 bp, including the pseudogen). Primers used were: sense 5' GAC TTG GGT CTC CCG AAC TGG GG-3'; anti-sense 5'CAA AGG AGA GTT GAG ATT GTA GTA-3'. The obtained products of each one of the challenged viruses were sequenced and compared among them. The differences were established using the CLUSTAL ® program. On the other hand, in order to identify differences in antigenic sites, an alignment of the amino acid sequences between the PV rabies reference strain and the skunk isolate amplified segments was made.

RESULTS AND DISCUSSION

In this study, we evaluated the most common rabies vaccines used in Mexico. For human being, Ministery of health mainly distributes the VERO cell vaccine, since its cost is lower in comparison with rabies human diploid cell vaccine (Table 1). For dogs and other domestic species is commonly used the inactivated vaccine (PV strain), this biological is extensively employed in official canine rabies campaigns in Mexico. On the other hand, we also evaluate the VR-G vaccine intramuscularly. This vaccine has been tested orally for efficacy and safety in foxes and in other wildlife confirming good results (Grosenbaugh *et al*., 2006; Blanton *et al*., 2007). As it can be observed, Vaccine A (recombinant vaccinia-rabies VRG) conferred a protection higher than 5.0 IU (International Units) against the challenge viruses in the three strains, this is consistent with other studies conducted years ago using

Table 1: Protection levels obtained with four vaccines against three Mexican wild variants of rabies (vampire bat, lynx and skunk)

Vaccine	Aerial cycle vampire bat	Terrestrial cycle lynx/fox	Skunk cycle divergent 19% genome difference	Price/dose
Wild animal used VRG recombinant Administered IM	>5	>5	>5	1.5 US$
Veterinary inactivated used adjuvated PV strain	2.36	3.4	4	0.5 US$
Human used diploid cells PM strain	4	4.3	4	35 US$
Human used VERO cells PM strain	8	4.5	7	25 US$

All results are represented as International Units; IM: Intramuscularly; US. American dollar

different routes (Fujii *et al*., 1994). Vaccine B (inactivated adjuvanted vaccine, PV strain) conferred to the vampire strain 2.36 IU, to the lynx/fox strain 3.4 IU and to the skunk strain 4 IU. According to the Mexican regulations, vaccines used in dog vaccination campaigns must have at least a titer of 2 IU. This is to prevent any deficiencies that might exist in the handling of the vaccines in the field, specifically a lost in the cold chain.

On the other hand, human diploid vaccine showed a good level of protection against the challenge of the wild rabies virus variants evaluated here, such as in other studies (Kulkarni *et al*., 2007), as it can be observed this vaccine conferred 4 IU against aerial cycle (vampire-bat); 4.3 IU against terrestrial cycle (lynx/coyote) and 4 IU against the skunk strain, these results are little lower in comparison with Vaccine D (human used vaccine produced in VERO cells), which conferred 8 IU for vampire strain, lynx/coyote 4.5 IU and 7 IU against skunk isolate (Table 1). Concerning to diploid cells vaccine, our results could be similar to other studies in which this vaccine conferred protection even against European Bat Lyssavirus (EBL) types 1 and 2 (Brookes *et al*., 2005). However, as it is known in American Continent only has been detected the Lyssavirus genotype 1 (Rabies) (Loza-Rubio *et al*., 1996; Nadin-Davis and Loza-Rubio, 2006; Velasco Villa *et al*., 2006).

Both biologicals (C and D) are extensively used all over the world. In Mexico, VERO cells vaccine is the most currently used by Mexican health institutions for human vaccination, especially because of its lower cost. Moreover, as we tested in this research, it gives an adequate protection against rabies originated by wild animals.

As it can be observed, all vaccines tested gave protection titers higher than 2 IU against all three challenge variants, which is the minimal required in Mexico, surpassing the requirements established by the WHO (1 IU for animal vaccines and 2.5 IU for humans) (Vodopja, 1988).

Figure 1 shows the time which took the animals to succumb using the dilution that killed between 50 and 70% of the mice with the different challenge viruses. For virus Mxbv 3137 (aerial cycle) isolated from a bovine, we used a dilution 10^{7} which represents 1.5 DL 50; for the Mxgm 3148 isolated from a lynx, we used a dilution 10^{6}

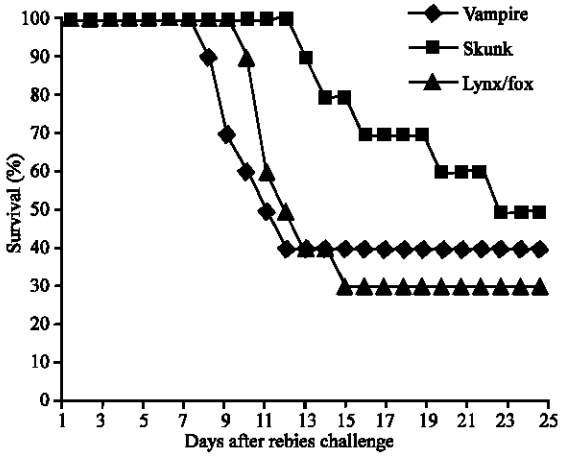

Fig. 1: Survival rates and incubation periods of the three divergent isolates

which represents 1.8 DL 50 and for the Mxsk 3136 isolated from a skunk, we used a dilution 10^{3} which represents 1 DL 50. The skunk isolate delayed significantly more in causing the death of mice and a higher concentration of virus was needed to cause the death of 50% of the challenged animals.

The 4 evaluated vaccines were efficient against the 3 variants evaluated here, including the skunk isolate challenge in spite of the differences showed in this study in which we used a region from G gene (Fig. 2). These differences have also been observed using another two genes from rabies virus, N and P genes (De Mattos *et al*., 1999; Nadin-Davis and Loza-Rubio, 2006; Velasco *et al*., 2006). In the skunk strain, it was also observed a difference of 2 amino acids in the analyzed segment of the G gene, which is directly involved in the immunologic response (site II, located between amino acids 53 and 61 of the rabies glycoprotein) (Prehaud *et al*., 1988). Apparently, genomic differences existing among the skunk strains and the others were not translated in significant antigenic differences. In fact, we observed that the skunk isolate was less virulent than the other challenge tested strains. Incubation periods of the disease were longer in animals challenged with skunk isolate and a higher concentration of this variant was needed to cause the death of 50% of the challenged animals, the same situation has been observed in other studies

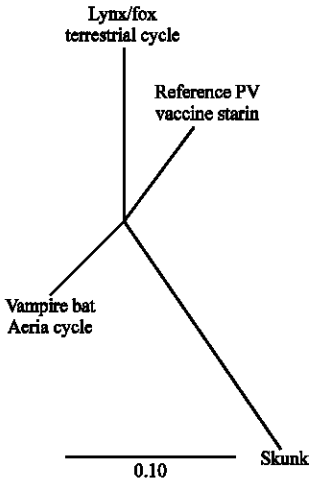

Fig. 2: Oligodendographe representing genomic differences among the rabies virus used. This was made using the CLUSTAL ® program (1 pb = 0.10) comparing a segment of approximately 600 pb between the rabies virus G and L genes

(Loza-Rubio *et al*., 2005). VR-G vaccine is currently administered by the oral route to foxes, raccoons and coyotes. The results obtained in this study using this vaccine by parenteral route should make this vaccine to be considered for future applications in veterinary medicine.

CONCLUSION

In conclusion, this is the first study made in Mexico to ascertain and certify that there is cross-protection of the most used vaccines and three divergent wild animal molecular variants of rabies virus.

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