

COMPARISON OF THE INTRANASAL AND INTRAMUSCULAR ROUTES OF VACCINATION OF ATTENUATED LIVE FOOT-AND-MOUTH DISEASE VIRUS TYPE O

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SHORT COMMUNICATION

Traditionally attenuated live foot-and-mouth disease (FMD) virus has been applied in Venezuela by the intramuscular route (3, 8).

Preliminary studies at the Pan American Foot-and-Mouth Disease Center (PAFMDC) showed that it might be feasible to immunize cattle by the intranasal route with attenuated live FMD virus vaccine (1, 5, 6). The advantage of such a procedure would be the multiplication of vaccine virus at the portal of entry of the infection, even in cattle which already have circulating virus neutralizing antibodies.

Concievably such a virus growth would also stimulate local defense mechanisms.

At the Institute of Veterinary Research in Maracay, Venezuela, four groups of 8 cattle, which were susceptible to virus O₁, were inoculated with the O₁ Campos attenuated strain presently used for vaccine production in Venezuela (2).

Two of the groups were inoculated with 5 ml of virus suspension by intranasal instillation and 2 groups were inoculated with a similar amount intramuscularly. For each route of inoculation a low and a high dose were used of 10^{5.2} and 10^{7.2} mouse ID₅₀ respectively.

The attenuated virus was suspended in 40% buffered glycerin.

Oesophageal-pharyngeal (OP) fluid samples (7) were collected at 1, 2 and 3 days after inoculation of the attenuated virus. At day 3 heparinized blood samples were collected.

Both the OP fluid samples and the blood sam-

ples were inoculated in 7 days old mice for virus isolation.

In the group inoculated intranasally with 10^{5.2} ID₅₀ of attenuated virus, O₁ virus could be isolated from 4 cattle.

In the group inoculated intranasally with 10^{7.2} ID₅₀, the OP fluid samples of 8 animals were positive to virus type O. Thus the "take rate" was the highest with the high virus dose.

Following inoculation of the attenuated virus, viremia was detected in one animal inoculated intranasally with 10^{7.2} ID₅₀ of virus; this animal was the only one which developed interdigital skin lesions.

Serum samples were collected at 0, 30, 60 and 90 days after vaccination. Antibody tests of serum against the O₁ Campos field strain were carried out using the microneutralization technique (4) or the mouse protection test (3).

The intranasal group inoculated with the highest dose, was the only group in which any significant serum conversion occurred (Table 1).

All cattle were exposed by intranasal instillation of 10^{7.1} mouse ID₅₀ of virulent O virus strain O₁ Cura 90 days after vaccination. Cattle were observed for 14 days. A blood sample for viremia assay was collected 3 days after exposure.

This virulent virus was previously tested by intranasal inoculation of 4 unvaccinated cattle similar to those of the experiment. These control cattle developed severe FMD 3-4 days after inoculation.

In the high-dose intranasal group, 4 of 8 cattle did not develop FMD lesions. One animal with a high mouse protection index only had a small superficial lesion on one foot (Table 2). All other cattle developed generalized FMD with the exception of one animal belonging to the intranasal group inoculated with 10^{5.2} ID₅₀ of attenuated virus.

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TABLE 1. Mean serum neutralization titers of cattle after intramuscular or intranasal inoculation of attenuated FMD virus O₁ at two different dose levels

Route	Dose ^a							
	10 ^{5.2}				10 ^{7.2}			
	Days after inoculation				Days after inoculation			
	0	30	60	90	0	30	60	90
Intramuscular	≤0.9	≤1.0	≤1.3	≤1.0	≤1.0	1.2	≤1.0	≤1.0
Intranasal	≤0.9	≤1.0	≤1.0	≤1.0	≤1.0	2.1	2.3	2.2

^aSuckling mouse ID₅₀ in 5 ml inoculum.

TABLE 2. Mouse protection index of cattle 90 days after intranasal inoculation of 10^{7.2} ID₅₀ attenuated FMD virus type O and results of intranasal challenge^a with 10^{7.1} ID₅₀ of virulent virus

Intramuscular			Intranasal		
MPI ^b	Viremia ^c	Clinical signs	MPI ^b	Viremia ^c	Clinical signs
0.0	+	Generalized	0.1	+	4 feet
0.6	+	"	2.7	-	4 feet, lip
0.0	+	"	0.0	-	4 feet
0.5	+	"	4.7	-	None
1.2	+	"	1.7	-	None
0.4	+	"	4.2	-	None
1.2	+	"	4.2	-	None
0.7	+	"	4.7	-	1 foot

^aAgainst FMD virus strain O₁ Cura.

^bMPI = Mouse protection index.

^cOnly tested at 3 days post-challenge.

All cattle of the intramuscular groups had viremia on day 3 after exposure. Of the low-dose and the high-dose intranasal groups respectively seven and one cattle were viremic at Day 3.

We may conclude that 10^{7.2} ID₅₀ of attenuated virus inoculated intramuscularly is not likely to afford an adequate level of protection against the O₁ Cura field strain.

Results of the intranasal inoculation are more favorable and confirm earlier observations (1, 5, 6) that the intranasal route may be a more suitable for the application of attenuated FMD virus to immunize cattle.

REFERENCES

1. AUGE DE MELLO, P.; GOMES, I.; SUTMÖLLER, P. Virus atenuado de la fiebre aftosa. I. Estudio preliminar sobre la replicación del virus y la respuesta inmunológica de bovinos después de la instilación intranasal de la cepa A₂₄ Cruzeiro. (Attenuated foot-and-mouth disease virus. I. Preliminary study of virus growth and immune response of cattle after intranasal instillation of strain A₂₄ Cruzeiro). (Not published).
2. BALESTRINI, J.; BERNAL, C.; JIMENEZ, J.M.; MALDONADO, A.; CASTAÑEDA, J.M.; GOMEZ, G. Ultimas observaciones sobre el comportamiento de las vacunas a virus vivo contra la fiebre aftosa.

- sa en Venezuela. *Revta. Vet. Trop.* 1 (1): 3-14, 1976.
3. CUNHA, R.G.; BAPTISTA Jr., J.A.; SERRÃO, U.M.; TORTURELLA, I. El uso de los ratones lactantes en la evaluación de los anticuerpos contra el virus de la fiebre aftosa y su significación inmunológica. *Gac. vet.*, B. Aires, 19 (110): 243-267, 1957.
 4. FERREIRA, M.E.V. Prueba de microneutralización para estudios de anticuerpos de la fiebre aftosa. (Microtiter neutralization test for the study of foot-and-mouth disease antibodies). *Bol. Centr. Panam. Fiebre Aftosa* 21-22: 17-20, 21-24, 1976.
 5. GOMES, I.; AUGÉ DE MELLO, P. Virus atenuado de la fiebre aftosa. III. Crecimiento del virus y respuesta inmunitaria a la instilación intranasal en bovinos de la cepa O₁ Campos. (Attenuated foot-and-mouth disease virus. III. Virus growth and immune response of cattle after intranasal instillation of strain O₁ Campos). (Not published).
 6. ROSENBERG, F.; GOMES, I. Virus atenuado de la fiebre aftosa. II. Crecimiento del virus y respuesta inmunitaria duradera a la aplicación intranasal en bovinos de la cepa A₂₄ Cruzeiro. (Attenuated foot-and-mouth disease virus. II. Virus growth and long-term immune response of cattle after intranasal application of strain A₂₄ Cruzeiro). (Not published).
 7. SUTMÖLLER, P.; COTTRAL, G.E. Improved techniques for the detection of foot-and-mouth disease virus in carrier cattle. *Arch. ges. Virusforsch.* 21:170-177, 1967.