INTRANASAL VACCINATION AND REVACCINATION OF CATTLE WITH FOOT-AND-MOUTH DISEASE ATTENUATED LIVE VIRUS VACCINE STRAIN O_1 CAMPOS

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SUMMARY

The cattle response to intranasal vaccination and revaccination with modified live virus vaccine type O was studied, using the challenge method with virulent virus and the determination of antibody titers. Two groups of animals free from foot-and-mouth disease antibodies were inoculated with $10^{6.5}$ and $10^{7.5}$ mouse ID_{50} of the modified live virus, respectively. It was shown that it is feasible to use the intranasal route of vaccination with this virus.

INTRODUCTION

Earlier experiments (*2, 8*) determined that it was possible to immunize cattle by the instillation of attenuated live foot-and-mouth disease (FMD) vaccine. In a preliminary experiment in Venezuela (*4*) two dose levels of vaccine ($10^{5.2}$ and $10^{7.2}$ mouse ID_{50}) of strain O_1 Campos were tested by the intramuscular and intranasal routes. It was shown that $10^{7.2}$ mouse ID_{50} by the intranasal route gave the best immune response in the vaccinated cattle.

In the present experiment the response to intranasal vaccination and revaccination with the same attenuated strain was studied in order to assess the potential usefulness of intranasal vaccination with strain O_1 Campos in the control of FMD in Venezuela.

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MATERIALS AND METHODS

Attenuated FMD virus

The O_1 Campos strain as presently used for vaccine production in Venezuela was used (*3*). The virus was suspended in 40% buffered glycerin (pH 7.6). The titer of this suspension was $10^{6.8}$ mouse ID_{50}/ml.

Virulent virus

For the cattle challenge, the virulent O_1 Cura field strain was used in the 3rd or 4th cattle passage in the laboratory.

Cattle

A group of 32 cattle was used in the vaccine experiment as well as 8 unvaccinated cattle which served as controls for the challenge. These cattle were 24-30 months old and originated from an FMD-free area in Venezuela and did not have FMD or VIA antibodies.

Sixteen cattle were inoculated with $10^{6.5}$ mouse ID_{50} per 5 ml dose (vaccine diluted 1:10) intranasally and another 16 cattle were inoculated similarly with 5 ml of the undiluted vaccine thus receiving a total dose of $10^{7.5}$ ID_{50}.

At 97 days post-vaccination (DPV) eight cattle from both groups together with 4 unvaccinated control cattle were exposed to the O_1 Cura field strain by the intranasal instillation of 2 ml virulent suspension containing $10^{5.4}$ ID_{50}. At this time the remaining 8 cattle of each group were revaccinated with the dose of attenuated vaccine which they had received originally and were exposed by intranasal route to $10^{4.0}$ of the O_1 Cura strain at 208 days post-revaccination (DPRV). Cattle were examined for clinical signs at 2, 3 and 7 days after each vaccination and on Days 3, 7 and 15 after challenge (DPC).

Collection of samples

Oesophageal-pharyngeal (OP) fluid samples were collected and processed according to routine procedures (*2*) at 2 and 3 days after vaccination and at approximately monthly intervals. Heparinized blood samples were collected at 2 and 3 DPV and DPRV and at 3 DPC.

Assay of samples

Each OP fluid or blood sample was inoculated in 8 unweaned 7-day old mice and in BHK cell monolayers. Dead mice or cell cultures with cytopathic effect were assayed by the complement fixing test for confirmation and determination of the virus type.

The neutralizing activity of the OP fluid was assayed in a final 1:4 dilution by the plaque reduction neutralization test as described (*1*). The serum antibodies were assayed by the microneutralization test (*6*) or by the mouse protection test (*5*).

RESULTS

Vaccination

Table 1 lists the results of cattle reactions after intranasal vaccination with two dose levels of the O_1 Campos attenuated virus.

With the $10^{6.5}$ ID_{50} dose of attenuated virus, 5 of the 16 vaccinated cattle had viremia only on Day 2 and one animal had viremia on Day 3. None of these cattle developed visible lesions.

After intranasal inoculation of $10^{7.5}$ ID_{50} of attenuated virus epithelial lesions were observed in 2 of 16 cattle which were viremic on Days 2 and 3. One animal which was viremic on Day 2 did not develop epithelial lesions.

FMD type O virus was detected in the OP fluid of all of the cattle 2 and 3 DPV and in 8 of the 32 cattle up to 90 days.

Revaccination

Reactions of the cattle after revaccination with the attenuated strain are summarized in *Table 2.* No viremia or lesions were observed. Of the 6 carriers found at 30 DPRV, 3 had already been carrying virus since the first vaccination. The other 3 carriers had shown no virus multiplication beyond 72 hours and their serum conversion was either weak or absent at the first vaccination. The number of carriers decreased soon after revaccination and only one of the newly established carriers remained positive up to 195 days.

TABLE 1. *Reaction of cattle[a] after intranasal vaccination of attenuated virus type O_1 strain Campos*

	Viremia – DPV			OPF – Days post-vaccination				
Dose mouse ID_{50}	2	3	Lesions	2	3	35	60	90
6.5	5[b]	1	0	16	16	10	11	4
7.5	3	2	2	16	16	6	8	4

[a] 16 cattle per group.

[b] Number of cattle positive per group.

DPV = Days post-vaccination.

OPF = Oesophageal-pharyngeal fluid.

TABLE 2. *Reaction of cattle[a] after intranasal revaccination of attenuated virus type O_1 strain Campos*

Dose	Viremia - DPRV	Lesions	OPF - Days post-revaccination						
	3		30	60	100	135	165	195	203
6.5	0[b]	0	3	2	0	0	0	0	0
7.5	0	0	3	2	1	1	0	1	0

[a] 8 cattle per group.
[b] Number of cattle positive per group.
DPRV = Days post-revaccination.
OPF = Oesophageal-pharyngeal fluid.

Antibodies

The results of the microneutralization tests are summarized in *Table 3.* The mean antibody titers remained at plateau levels from 30 DPV until revaccination at 97 days. At 30 DPRV slight increase of the mean antibody titers was observed in the group revaccinated with 7.5 ID_{50} of the attenuated virus as a result of the serum conversion of some previously negative cattle as well of a booster of some of the previous antibody titers. No significant difference was noted between the antibody response of the groups vaccinated with the 2 different dose levels of the attenuated strain.

TABLE 3. *Microneutralization titers of cattle after intranasal vaccination and revaccination with attenuated virus vaccine strain O_1 Campos against the Venezuela field strain O_1 Cura*

	Vaccine	
	Dose $10^{6.5}$ ID_{50}	Dose $10^{7.5}$ ID_{50}
Days post-vaccination		
0	$\leqslant 1.0$	$\leqslant 1.0$
30	2.2 ± 0.88[a]	2.3 ± 0.95
60	2.3 ± 0.78	2.1 ± 0.86
97	2.2 ± 0.69	2.2 ± 0.84
Days post-revaccination		
30	2.5 ± 0.59[b]	2.9 ± 0.73
60	2.5 ± 0.67	2.6 ± 0.58
100	2.3 ± 0.69	2.4 ± 0.43
135	2.1 ± 0.59	2.5 ± 0.39
195	2.2 ± 0.51	2.4 ± 0.51
203	2.1 ± 0.65	2.3 ± 0.68

[a] Mean and standard deviation of the mean (16 cattle).
[b] Mean and standard deviation of the mean (8 cattle).

The neutralization tests with the homologous O_1 Campos strain gave very similar results (not mentioned in the table).

The data in Table 5 show that the serum antibodies and the neutralizing activity of the OP fluid at 208 DPRV were lower than those at 90 DPV. In fact little influence of the revaccination was observed on the neutralization activity of the OP fluid, and activity decreased from 135 DPRV onward.

Challenge

The 8 non-vaccinated control cattle (not shown in the table) had viremia and starting clinical signs on Day 3 and developed severe FMD. Results of the challenge test at 90 DPV are shown in *Table 4*. On Day 3 of the challenge only 3 of the 16 vaccinated cattle which had received only one dose of attenuated virus were viremic and 2 of those had starting lesions. By Day 7 a total of 7 cattle had lesions. The cattle which developed FMD lesions included all of those with low antibody titers and 2 with high antibody titers.

Table 5 summarizes the results of the challenge at 208 days DPRV. No viremia was detected in any of the cattle. Only one animal of the group vaccinated twice with $10^{6.5}$ ID_{50} attenuated virus developed a foot lesion on Day 5. Three more cattle with lesions were detected at examination 9 DPC.

DISCUSSION

Previous work (*4*) showed a considerable difference in response after intranasal inoculation of $10^{5.2}$ and $10^{7.2}$ mouse ID_{50} of attenuated live FMD type O_1 virus; the lower dose proved less effective in infecting the cattle and this resulted in low serum neutralizing titers, and absence of protection against virulent virus exposure. These differences in response were not observed in the present study using $10^{6.5}$ and $10^{7.5}$ mouse ID_{50} as the inoculum of attenuated virus, respectively.

TABLE 4. *Results of cattle challenged with field strain O_1 Cura at 97 DPV*

Dose $10^{6.5}$ mouse ID_{50}					Dose $10^{7.5}$ mouse ID_{50}				
Reaction					Reaction				
Viremia[a]	Lesions	MNT	MPT	OP/PR	Viremia[a]	Lesions	MNT	MPT	OP/PR
+	T, 4F[a]	<1.2	0.0	76	–	– 4F	<1.2	0.0	73
+	T, 4F[a]	1.5	0.0	99	–	– 3F	1.9	0.4	95
–	T, 4F	3.0	4.5	100	–	– –	2.6	4.0	100
–	– 4F	2.1	3.5	99	+	T, 4F	<1.2	0.0	90
–	– –	2.4	5.5	100	–	– –	3.5	4.8	100
–	– –	3.0	5.1	100	–	– –	2.6	4.8	100
–	– –	2.3	2.0	99	–	– –	2.9	4.8	99
–	– –	2.3	3.9	100	–	– –	2.6	2.8	93

[a]Day 3.
DPV = Days post-vaccination.
MNT = Microneutralization test.
MPT = Mouse protection test.
OP/PR = Plaque reduction test; percentage of plaque reduction by OP fluid in a final dilution of 1:4.

TABLE 5. *Results of cattle challenged with field strain O_1 Cura at 208 DPRV*

Dose $10^{6.5}$ mouse ID_{50}					Dose $10^{7.5}$ mouse ID_{50}				
Reaction					Reaction				
Viremia[a]	Lesions	MNT	MPT	OP/PR	Viremia	Lesions	MNT	MPT	OP/PR
–	4F[a]	< 1.2	2.6	65	–	1F[b]	1.5	1.5	78
–	4F[b]	1.8	2.0	91	–	1F[b]	2.3	4.4	83
–	–	2.3	1.3	100	–	–	2.7	3.3	98
–	–	2.3	3.0	70	–	–	2.1	2.8	89
–	–	3.0	1.5	89	–	–	2.4	1.0	72
–	–	1.5	2.2	61	–	–	1.8	1.8	56
–	–	3.0	3.3	84	–	–	2.3	3.8	100
–	–	2.3	2.8	62	–	–	3.6	4.5	100

[a]Day 5. [b]Day 9.
DPRV = Days post-revaccination. MNT = Microneutralization test. MPT = Mouse protection test.
OP/PR = Plaque reduction test; percentage of plaque reduction by OP fluid in a final dilution of 1:4.

The O_1 strain used retains a degree of pathogenicity after intranasal inoculation. Of the 32 cattle inoculated 2 showed interdigital skin lesions. In accordance with earlier observations (*4*), the development of lesions appears to occur primarily in intranasally inoculated cattle which become viremic. It appears that the longer lasting the viremia the greater the chances of the development of lesions.

No viremia or pathogenicity was observed after revaccination of the remaining cattle. In fact very little response could be observed in the cattle which had shown virus multiplication and serum conversion after the first vaccination. There was only a slight short-lived booster of the serum neutralization titers, and their carrier rate decreased, until all original carriers were negative after 60 days. The 3 new carriers after revaccination were all cattle which had not responded well at the first vaccination.

The lack of response to the revaccination of the majority of cattle may be an indirect indication of their resistance against exposure to virulent virus.

In cattle recovered from virulent virus exposure, neutralizing activity of the OP fluid reaches its peak at 2-3 months followed by a gradual decrease, which is probably related to the progressive elimination of the carriers in the group (*7*). It appears from the present study that this response to attenuated virus infection is similar to that of virulent virus infection.

The present study confirms earlier works on the use of the intranasal route for FMD live virus vaccination (*2, 4, 8*) which showed that it is feasible to use this route to protect cattle against virulent virus. The effective dose by the intranasal route probably is lower than the dose of attenuated virus required for the intramuscular route (*4*).

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