PRELIMINARY OBSERVATIONS ON PHARYNGEAL VIRUS GROWTH AND VIREMIA AFTER INTRADERMOLINGUAL INOCULATION OF CATTLE WITH FOOT-AND-MOUTH DISEASE VIRUS

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SUMMARY

Foot-and-mouth disease virus growth was studied in vaccinated and unvaccinated cattle after inoculation of the tongue epithelium. Unvaccinated cattle were already viremic by 6-12 hours after inoculation. Earliest pharyngeal virus growth also occurred at that period. The results indicate that the pharyngeal virus growth likely is a consequence of direct infection of upper respiratory track. The early viremia in the unvaccinated cattle indicate a hematogenous infection of other sites at the time of tongue inoculation.

INTRODUCTION

Titration of foot-and-mouth disease (FMD) virus in the tongue epithelium of cattle (4), has been used extensively in FMD research. It is generally assumed that virus growth occurs at the injection sites (primary lesions) followed by viremia and generalization (secondary lesions). However, with intradermolingual (IDL) inoculation it is likely that also the upper respiratory tract becomes infected by aerosolation and spraying during the operation or by backflow of virus suspension from the inoculation site. The importance of this infection site was noted by McVicar and Sutmöller (5) who showed that within 6-12 hours the virus would reach high titers in the pharyngeal area after intranasal instillation of FMD virus.

On the other hand some of the virus suspension might also be deposited slightly less superficially than intended and reach the circulatory system. In that case the virus could be transported to other sites of virus replication independent of the tongue lesions developing at the inoculation sites.

The present study was made to determine if pharyngeal growth would occur and what the viremia patterns would be after IDL inoculation of cattle in the course of a vaccine potency control test (2).

MATERIALS AND METHODS

Cattle

Six unvaccinated crossbred Zebu steers originating from a farm on which FMD had not occurred for several years, were used. They were previously tested for the absence of protective and neutralizing antibodies. Four of the steers were vaccinated with an acetylethyleneimine inactivated FMD vaccine adjuvanted with aluminum hydroxide-saponin. They were used for a potency test (2) 21 days later. The 2 unvaccinated cattle served as controls in the test.

Virus

FMD virus, subtype O_1 strain Campos in bovine tongue epithelium was used. Ten-fold serial dilutions were made in Earle's salt solution (ESS). The tongue epithelium of each of the vaccinated steers was inoculated with 0.1 ml at 4 sites with each of 4 dilutions which contained approximately 10 to 10,000 bovine ID_{50}/ml. The control steers were given the dilution containing 1 to 1,000 bovine ID_{50}/ml by the same route.

The virus was inoculated under general anesthesia using 40% chloral hydrate at 0.2 ml/kg body weight.

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Clinical observation and collection of samples

The cattle were closely examined at 44 hours post-inoculation to score the tongue lesions, and at 3, 5 and 7 days post-inoculation to examine the feet.

Oesophageal pharyngeal (OP) fluid samples were collected at hourly intervals up to 6 hours, then at 2 hourly intervals up to 12 hours, and at 24, 30, 48, 72 hours.

The OP fluid samples were diluted immediately with equal part of ESS 1,000 IU penicillin, 1 mg streptomycin and 125 IU fungizon*/ml. Heparinized blood samples were collected at 6, 12, 24, 30, 48 and 72 HPI. All samples were stored at −70°C until testing. Before virus inoculation, sera were collected for antibody assay.

Virus assay

Infectivity titers were determined by plaque assay using IB-RS-2 monolayers in 6 cm Petri dishes and karaya gum overlay as described by Augé (1). The infectivity titers were expressed as $\log_{10}$ PFU/ml of blood or of undiluted OP fluid.

Antibody assay

Pre-exposure sera were tested by the mouse protection test according to the method described by Cunha *et al.* (3) with the results expressed as the mouse protection index.

RESULTS

The pre-challenge results of the mouse protection tests are listed in Table 1, together with the results of the clinical observations.

The two unvaccinated cattle had tongue lesions at 2 DPI and developed vesicles on all feet. The vaccinated cattle all had circulating antibodies and had tongue lesions usually only at the sites with the higher virus doses. Foot lesions were absent in the vaccinated cattle.

Table 2 lists the virus titers of the blood. No virus could be detected in the blood of the vaccinated cattle. The 2 unvaccinated steers were already viremic at 6 and 12 hours, respectively.

Virus titers of the OP fluid are listed in Table 3. No virus could be detected in the 1-4 hour samples of steer 1 or in the 1-8 hour samples of steer 2. Virus growth in these two unvaccinated steers followed a course quite similar to those described by McVicar and Sutmöller (5) for intranasally inoculated cattle. The relatively long lag period could point to a low level of initial infection of the pharyngeal area (5).

In vaccinated cattle virus growth was first seen between 6-10 hours. The lag period was longer but the virus titers comparable to those found by McVicar and Sutmöller (5), using $10^5 - 10^7$ PFU intranasally in vaccinated cattle. In this experiment a total of approximately 5×10^2 bovine ID_{50} were used for the IDL inoculation of each of the controls and 5×10^3 bovine ID_{50} in the vaccinated cattle.

Poorest growth was observed in steer 3. With the exception of this steer the infectivity of the OP fluid reached levels of 10^4 PFU/ml even though steer 6 had no tongue lesions. In this preliminary experiment there was no relationship between the extent of the tongue lesions or antibody titers and the degree of pharyngeal virus growth in the vaccinated cattle.

DISCUSSION

The growth of FMD virus in the pharyngeal area of these IDL inoculated cattle probably resulted from direct inoculation of the upper respiratory tract, since the hematogenous route is unlikely to occur in vaccinated cattle with circulating antibodies.

The high virus titers reached in the pharyngeal area shortly after IDL inoculation independent of antibody levels indicate that this route of infecting an animal is much more complex than usually has been assumed.

Also the early isolation of virus from the blood of unvaccinated steers is noteworthy. At that time, the growth in the pharyngeal area

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had just started and the tongue epithelium, was grossly normal. The amount of virus released in the pharyngeal area or from the inoculated sites on the tongue would probably be insufficient to account for the maintenance of a viremia of approximately 100 PFU/ml of blood (6). Such virus titers indicate that after the IDL inoculation virus entered the circulation probably at the time of inoculation followed by virus production in additional locations. A similar situation was found after intravenous infection of cattle with FMD virus, and it was suggested that the increase in blood titers shortly after intravenous infection could be caused by virus replication in the germinative layers of the skin or mucous membranes (7).

Inhalation of the virus and a deep lung infection (7) cannot be excluded, particularly since the animals were anesthetized during the inoculation.

TABLE 1. *Pre-challenge mouse protection indices (MPI) of cattle exposed to foot-and-mouth disease virus O_1 by IDL inoculation and the results of clinical observations*

Cattle No.	MPI	Tongue lesions					Foot Lesions
		10^4*	10^3	10^2	10^1	10^0	
1	0.4	–	4	4	3	1	4
2	0.5	–	4	4	4	3	4
3	4.2	2	1	0	0	–	0
4	2.3	4	2	3	4	–	0
5	4.2	4	4	2	0	–	0
6	3.8	0	0	0	0	–	0

* Virus dose/ml.

NOTE: Cattle 1 and 2 controls not vaccinated.
Cattle 3, 4, 5 and 6 vaccinated.

TABLE 2. *Infectivity titers of blood of cattle inoculated IDL with foot-and-mouth disease virus type O_1*

Hours post-inoculation	Cattle No.					
	1	2	3	4	5	6
0	–	–	–	–	–	–
6	1.0*	–	–	–	–	–
12	2.6	1.8	–	–	–	–
24	2.6	2.3	–	–	–	–
30	2.8	2.1	–	–	–	–
48	3.1	2.1	–	–	–	–
72	1.4	2.1	–	–	–	–

* Log_{10} PFU/ml.

NOTE: Cattle 1 and 2 controls not vaccinated.
Cattle 3, 4, 5 and 6 vaccinated.

TABLE 3. *Infectivity titers of oesophageal pharyngeal fluid from cattle inoculated IDL with foot-and-mouth disease with type O_1*

Hours post-inoculation	Cattle No.					
	1	2	3	4	5	6
0	–	–	–	–	–	–
1	–	–	–	–	–	–
2	–	–	–	–	–	–
3	–	–	–	–	–	–
4	–	–	–	–	–	–
5	1.0*	–	–	–	–	–
6	2.1	–	–	–	0.9	0.9
8	4.4	–	1.1	–	–	–
10	3.3	0.9	1.4	1.4	2.4	–
12	3.8	0.9	1.4	1.0	3.0	1.4
24	5.8	6.0	0.9	2.2	4.9	2.4
30	5.6	5.8	–	2.3	4.4	2.0
48	4.6	6.0	–	4.0	4.9	2.0
72	5.6	5.2	–	3.8	3.0	4.2

* Log_{10} PFU/ml Oesophageal pharyngeal fluid.

NOTE: Cattle 1 and 2 controls not vaccinated.
Cattle 3, 4, 5 and 6 vaccinated.

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