A Research Note
Persistence of Foot-and-Mouth Disease Virus in Dried Casein

H. R. CUNLIFFE*, J. H. BLACKWELL and J. S. WALKER

Plum Island Animal Disease Center, Science and Education Administration
U.S. Department of Agriculture, Greenport, New York 11944

(Received for publication February 17, 1978)

ABSTRACT

Dried casein produced from pasteurized milk of dairy cows infected with foot-and-mouth disease (FMD) virus retained infectivity for cattle in one of seven tests for 42 days of storage at 25°C. Thus, infectious FMD virus can persist after pasteurization of the milk at 72°C for 15 sec., acid precipitation and washing of casein, followed by drying of the casein in a hot air flow and conversion to sodium caseinate.

Results of a previous study (3) indicated that foot-and-mouth disease (FMD) virus could survive temperature and acid conditions used to produce casein and sodium caseinate with infective cow's milk. Limited data in that study also suggested that commercial casein drying procedures did not destroy FMD virus infectivity.

The potential of such products to initiate an FMD epizootic in susceptible livestock is of particular concern in FMD-free countries. Therefore, in the following study, the persistence of infectious FMD virus in dried casein was assessed under controlled storage conditions.

MATERIALS AND METHODS

Details of milk and casein production, animal care, cell culture procedures, infectivity assays and viruses used in this study appear in a previous report (3). As near as possible, production and storage procedures were designed to simulate those most often in the drug industries. Seven batches of dried casein were produced from skimmed milk of five dairy cows infected with FMD virus type A, subtype 3, strain Mecklenburg. All milk was obtained 1 day after infection, usually before clinical signs of FMD were evident.

Damp casein was dried in a stainless-steel vertical column drier. The casein was held on a fine-mesh brass screen tray vibrated by a eccentric cam on a motor-driven flexible drive shaft attached to the tray. Air (30-28°C) was blown upward through the column for 40 min. Then the input air was heated so that the tray was 65-68°C for 10 min. After drying, the casein contained 8-10% moisture by dry-weight analysis.

Dried casein was coarsely ground with mortar and pestle, after which 2-g samples were gasket-sealed over approximately 2 ml of ambient air in screw-cap vials and stored away from direct light at room temperature (20-25°C) until used.

For infectivity testing, 2-g samples of dried casein were finely ground with mortar and pestle and dissolved in 18 ml of F-14 medium (Grand Island Biological Co., Grand Island, New York 14072). The mixture was stirred magnetically while pH was maintained between 7.0 and 8.0 by careful addition of 1 N NaOH. Dried casein slowly dissolved to sodium caseinate as the pH was raised to 8.0. In an effort to assure total solution, the slurry was repeatedly expelled through an 18-gauge needle.

After 1 day storage of dry casein, persistence of FMD virus in the derived sodium caseinate was tested in two steers. Six steers were used in all other sampling times. Each animal was inoculated with 2 ml of liquid sodium caseinate into 20 sites in the epithelium of the dorsal surface of the tongue and with 8 ml inoculated intramuscularly. Thus, each steer received 1 g of dried casein that was obtained from about 40 ml of skim milk. Six steers were inoculated at storage times of 21, 42, and 84 days. Steers were observed for 14 days postinoculation (DPI), and serum from non-reactors was assayed for FMD virus antibodies by virus neutralization procedures (2).

RESULTS AND DISCUSSION

Milk yield did not decrease nor were any other signs of disease seen in the dairy cows at 1 DPI other than fever in one of the five cows. However, the amount of FMDV recovered from their skim milk before pasteurization (72°C for 15 sec) ranged from 5.5 to 6.4 log 10 plaque-forming units/ml (Table 1).

Four of the seven batches of dried casein contained FMD virus that was infectious for steers at 1 day of storage. However, only one (Batch No. 2, Table 1) of the four positive batches was infectious for steers after 21 days in storage. This batch remained infectious for steers through 42 days of storage at ambient temperature. Neutralizing antibody for FMD virus was not detected in 14-DPI serum from steers clinically negative for FMD.

Interestingly, only one of four batches of dried casein from the same pool of skim milk retained infectious FMD virus beyond 1 day of storage. This finding suggests that very minor deviations in techniques may affect survival of infectious FMD virus in the final product. In our studies, time, temperature, pH and moisture were carefully controlled to insure uniform conditions.

Observations in previous studies (1, 3) indicated a considerable difference in FMD virus-sensitivity between...
TABLE 1. Infectivity of dried casein from foot-and-mouth disease virus-infected cows.

<table>
<thead>
<tr>
<th>Casein batch No.</th>
<th>Raw skim milk titer&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Pasteurized skim milk titer&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Cattle results after days in dry storage&lt;sup&gt;c,d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>21</td>
<td>42</td>
</tr>
<tr>
<td>1</td>
<td>6.4</td>
<td>&lt;1.0</td>
<td>0/2 NT NT NT</td>
</tr>
<tr>
<td>2</td>
<td>6.4</td>
<td>&lt;1.0</td>
<td>2/2 5/6 5/6 0/6</td>
</tr>
<tr>
<td>3</td>
<td>6.4</td>
<td>&lt;1.0</td>
<td>0/2 NT NT NT</td>
</tr>
<tr>
<td>4</td>
<td>6.4</td>
<td>&lt;1.0</td>
<td>2/2 0/6 NT NT</td>
</tr>
<tr>
<td>5</td>
<td>5.7</td>
<td>NT&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0/6 NT NT NT</td>
</tr>
<tr>
<td>6</td>
<td>5.5</td>
<td>&lt;1.0</td>
<td>1/2 0/6 NT NT</td>
</tr>
<tr>
<td>7</td>
<td>6.2</td>
<td>1.6</td>
<td>1/2 0/6 NT NT</td>
</tr>
</tbody>
</table>

<sup>a</sup>Casein batches 1 through 4 from the morning milking of one cow.
<sup>b</sup>Casein batch 5 was from a mixture of morning and evening milk of the same cow (batches 1-4).
<sup>c</sup>Casein batch 6 was from the pooled milk of three cows.
<sup>d</sup>Casein batch 7 was from the milk of another cow.
<sup>1</sup>Log<sub>10</sub> plaque forming units/ml.
<sup>2</sup>Two cattle used for 1-day storage assays; six cattle used thereafter; ratio = number of cattle positive/number inoculated.
<sup>d</sup>NT = not tested.

cell cultures and in vivo bovine tongue epithelium. Although cell cultures did not indicate infectious FMD virus in milk samples, Blackwell and Hyde (1) demonstrated infectious FMD virus when sample aliquots were inoculated into bovine tongue epithelium. Similarly, data shown in Table 1 (Batches No. 2 and 6) indicate a negative response in pasteurized skim milk assayed in cell cultures, yet dried casein produced from the same milk was infectious for steers.

Although responses shown in Table 1 indicate that the steers developed FMD, the disease was initiated by primary vesicles that developed at only one to five of the 20 sites inoculated on each tongue. These data suggest that casein thus dried and stored may contain a very small quantity of infectious FMD virus that is close to the threshold for detection in cattle.

ACKNOWLEDGMENTS

The authors are indebted to Mr. R. Trower and Mr. T. Sawicki for able technical assistance in this study.

REFERENCES