Survey of Soft and Semisoft Cheese for Presence of Fecal Coliforms and Serotypes of Enteropathogenic *Escherichia coli*

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ABSTRACT

Soft and semisoft cheese varieties including Camembert, Brie, brick, Muenster, and Colby were analyzed for fecal coliforms and serotypes of enteropathogenic *Escherichia coli* (EEC). Analysis for EEC was done using both direct streak and two enrichment procedures so that atypical strains, if present, would be recovered. Of 106 samples collected during the summer of 1977, 57.5% contained less than 100 fecal coliforms/g and 17.0% contained over 10,000 fecal coliforms/g. Serotypes of EEC were not detected in any of these samples.

Much of the past research concerning coliforms in cheese dealt with prevention of gassy defects and with significance of coliform contamination as an index of unsanitary manufacturing practices (2,10). However, since the occurrence of an outbreak of foodborne disease caused by enteropathogenic *Escherichia coli* (EEC) and associated with consumption of soft-ripened cheese (10), presence of coliforms in market cheese has taken on added significance. EEC have the ability to grow during the manufacture of soft and semisoft cheese and to survive in the ripened product (5,6). EEC, found as a natural contaminant in soft-ripened cheese, can grow in the ripened cheese at refrigeration temperatures (5).

An improved screening procedure for serotypes associated with EEC was developed by Mehlman et al. (11). Previous methods were inadequate because they failed to detect slow-lactose fermenting strains of EEC and strains which failed to grow at the elevated temperatures used (11,13). We used the improved methodology and thus we hope to present a more accurate estimate of the extent of EEC contamination in market cheese. Additionally, information is reported on fecal coliforms in cheese.

MATERIALS AND METHODS

**Sample collection**

Samples were collected from retail grocery and cheese stores in Madison, Wisconsin and vicinity during the summer of 1977. Both sliced and unsliced cheeses were selected, and representative samples of a variety of brands were obtained. Samples were refrigerated in the laboratory for a maximum of 6 days before analyses were done. Types of soft-ripened cheese that were sampled included Camembert and Brie. The semisoft cheeses that were sampled included brick, Muenster, Colby, farmer's cheese, and Monterey Jack.

**Analysis for fecal coliforms**

Numbers of fecal coliforms were estimated by a 3-tube Most Probable Number (MPN) procedure as described by Fishbein et al. (4) in the Compendium of Methods for the Microbiological Examination of Foods.

**Analysis for serotypes of enteropathogenic E. coli**

Analysis for serotypes of EEC was done by the method described by Fishbein et al. (4) which is similar to that proposed by Mehlman et al. (11). This method is qualitative and involves two separate enrichments, as well as a direct streaking of the sample.

Two 25-g portions of the sample were blended with 225 ml of MacConkey broth (Difco) and 225 ml of nutrient broth (Difco) each for 30 sec using a Waring blender. The 1:10 dilution in nutrient broth was then streaked onto Eosin Methylene Blue (EMB, Difco) and MacConkey (Difco) agars, and these plates were incubated at 35 C for 24 h. After incubation, 10 typical E. coli colonies were picked from the EMB agar and 10 colonies unable to use lactose were picked from the MacConkey agar. These were placed on Blood Agar Base (Difco) and then screened serologically. A slide agglutination test with polyvalent A, B, and C antisera (Difco) was used.

Enrichments involved incubation of the MacConkey broth mixture for 20 h at 35 C, and then transferring one loopful of this mixture to 30 ml of lauryl sulfate tryptose broth (LST, Difco). This was incubated at 44 C for 20 h. The nutrient broth mixture was incubated for 6 h at 35 C, then one loopful was transferred to 30 ml of enteric enrichment broth (EE broth, Difco). This was then incubated at 41.5 C for 18 h.

The LST and EE enrichments were neutralized with sterile 10% NaHCO₃ before serological screening. Slide agglutination tests were done on the enrichments using polyvalent A, B, and C antisera. LST enrichments with positive agglutination tests were streaked onto EMB agar and EE broth enrichments with positive agglutination tests were streaked onto EMB and MacConkey agars for isolation of individual colonies. Isolates thought to be *E. coli* and obtained from enrichment cultures and from direct streak plates were subjected to biochemical tests necessary for identification of *E. coli* as described by Mehlman et al. (11) and Fishbein et al. (4).

RESULTS AND DISCUSSION

**Occurrence of fecal coliforms**

One hundred and six different retail samples of cheese were analyzed for EEC and fecal coliforms. Twenty four
different brands of cheese were obtained. The distribution of fecal coliforms in these samples is given in Table 1. Samples of soft cheese included imported brands, these being from France and Denmark. Five of the imported samples of soft-ripened cheese were processed in cans, and these all had less than 10 fecal coliforms/g. The five samples of soft-ripened cheese which contained over $10^4$ fecal coliforms/g were all from the same domestic manufacturer from whom we obtained a total of 7 samples. Two of these samples contained about $10^4$ fecal coliforms/g and appeared gassy. However, they could have been considered edible by some consumers. Fortunately, serotypes of EEC were not isolated from these samples. Six different brands of soft-ripened cheese were analyzed; only one brand yielded samples exceeding 1000 fecal coliforms/g.

TABLE 1. Distribution of fecal coliforms in soft-ripened and semisoft market cheese

<table>
<thead>
<tr>
<th>Sample description</th>
<th>No. of samples</th>
<th>MPN per gram</th>
<th>10</th>
<th>10-100</th>
<th>101-1000</th>
<th>10,000</th>
<th>&gt; 10,000</th>
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<td></td>
<td></td>
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<tr>
<td>Soft-ripened1</td>
<td>25</td>
<td>60</td>
<td>10</td>
<td>10-100</td>
<td>101-1000</td>
<td>10,000</td>
<td>&gt; 10,000</td>
</tr>
<tr>
<td>Sliced1</td>
<td>17</td>
<td>64</td>
<td>10</td>
<td>10-100</td>
<td>101-1000</td>
<td>10,000</td>
<td>&gt; 10,000</td>
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<tr>
<td>Whole1</td>
<td>106</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brick</td>
<td>28</td>
<td>32.1</td>
<td>21.4</td>
<td>14.3</td>
<td>14.3</td>
<td>17.9</td>
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<tr>
<td>Colby</td>
<td>26</td>
<td>42.3</td>
<td>19.2</td>
<td>15.4</td>
<td>3.8</td>
<td>19.2</td>
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<tr>
<td>Muenster</td>
<td>18</td>
<td>38.9</td>
<td>5.6</td>
<td>16.7</td>
<td>22.2</td>
<td>16.7</td>
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<tr>
<td>Misc.1</td>
<td>9</td>
<td>22.2</td>
<td>22.2</td>
<td>22.2</td>
<td>33.3</td>
<td>0</td>
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<td>Total</td>
<td>81</td>
<td>35.8</td>
<td>17.3</td>
<td>16.0</td>
<td>14.8</td>
<td>16.0</td>
<td></td>
</tr>
<tr>
<td>Sliced1</td>
<td>17</td>
<td>35.3</td>
<td>23.5</td>
<td>11.8</td>
<td>11.8</td>
<td>17.6</td>
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</tr>
<tr>
<td>Whole1</td>
<td>64</td>
<td>35.9</td>
<td>15.6</td>
<td>17.2</td>
<td>15.6</td>
<td>15.6</td>
<td></td>
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<tr>
<td>Total soft</td>
<td>106</td>
<td>41.5</td>
<td>16.0</td>
<td>14.2</td>
<td>11.3</td>
<td>17.0</td>
<td></td>
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<td>and semisoft</td>
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</tbody>
</table>

1Includes Camembert and Brie varieties
2Includes farmers' and Monterey Jack varieties
3Sliced brick, Colby, and Muenster varieties
4Unsliced semisoft cheese

Distribution of fecal coliforms in semisoft cheese is given in Table 1. Sixteen percent of the samples contained over $10^4$/g and 52% contained less than 100/g. Distribution of fecal coliforms among the different varieties was similar. Also, the distribution was similar for sliced and whole cheese. Nine samples of semisoft cheese contained over 24,000 fecal coliforms/g with one as high as $2 \times 10^5$/g. These samples included aged brick (Bierkaese), mild brick, Colby, and Muenster varieties. Several samples with over $10^4$ fecal coliforms per gram were gassy, but most were not.

The overall level of fecal coliform contamination we observed was much higher than that found by Collins-Thompson et al. (1) and Jones et al. (9). Collins-Thompson et al. (1) reported only 0.8% of semisoft and 2.1% of soft cheese samples contained over 1600 fecal coliforms/g. A possible explanation for this difference is that the occurrence of fecal coliforms in pasteurized dairy products is seasonal, with greatest numbers appearing during the summer months (9). Our study was done exclusively during the warmest (heat wave) part of the summer. Thus our survey only gives an estimate of contamination occurring in cheese at a particular location and at a specific time of year. However, even with our small sample size, the high levels of contamination we found, whether normal or not, are still cause for concern, because they indicate that an undesirable situation existed which could be repeated. The actual number of E. coli present may be lower than the estimated fecal coliform population. Hall et al. (8) found that only 40.4% of EC-positive tubes from MPN tests for fecal coliforms in market foods contained E. coli.

Occurrence of EEC serotypes

No serotypes of enteropathogenic E. coli were isolated from the cheese samples. This is fortunate considering the large amount of coliform contamination present in the samples. This result is also encouraging because the EEC enrichment procedures used were designed to recover slow-lactose fermenting and temperature-sensitive as well as typical EEC. However, the sensitivity of the methods used for these tests has not been determined. Other studies have also found a very low incidence of EEC serotypes in pasteurized dairy products (9,14,15), though their methods might not have recovered atypical strains (13). Jones et al. (9), in a survey of cheeses in Canadian pasteurized dairy products, found three EEC serotype isolates which was 2% of the E. coli isolates examined. The low pathogenic potential of E. coli isolated from foods is discussed by Mehlman et al. (12). Of E. coli isolates from cheese involved in recent EEC foodborne gastroenteritis outbreaks, only 14% were invasive and 2% toxigenic.

Goldschmidt and DuPont (7) as well as Mehlman et al. (12) have presented evidence that pathogenicity and serotype are not as closely correlated as once thought. The fact that serotypes of EEC were not isolated from our samples does not completely prove their safety for at least two reasons: (a) EEC not belonging to the serotypes for which we tested could be present, and (b) EEC strains could have been present as a small fraction of the E. coli population in a particular sample, and during enrichment could have been overgrown by the other strains. Cheese containing $10^3$ EEC/g has been implicated in causing foodborne illness (10), so some of our samples would contain close to hazardous levels of coliforms if EEC strains had predominated.

Large numbers of coliforms in cheese can result from excessive post-pasteurization contamination of milk, or excessive growth during manufacture prompted by low starter culture activity (5). The possibility of coliform growth occurring because of inadequate refrigeration during retail handling of cheese should be investigated. Though EEC contamination in pasteurized dairy products may be rare, the common occurrence of large numbers of coliforms in soft and semisoft cheese represents a potential hazard. This indicates a need for more concern on the part of the dairy industry to prevent contamination of cheese with coliforms and thus to prevent additional outbreaks of foodborne illness caused by EEC.
ACKNOWLEDGMENT

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REFERENCES


Amendment to the
3-A Sanitary Standards for Centrifugal and Positive Rotary
Pumps for Milk and Milk Products, Number 02-06

Number 02-07

Formulated by
International Association of Milk, Food, and Environmental Sanitarians
United States Public Health Service
The Dairy Industry Committee

It is the purpose of the IAMFES, USPHS, and DIC in connection with the development of the 3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Milk pump specifications heretofore or hereafter developed which so differ in design, material, construction, or otherwise, as not to conform with the following standards, but which in the manufacturer's or fabricator's opinion are equivalent or better may be submitted for the joint consideration of the IAMFES, USPHS, and DIC at any time.

The 3-A standards for centrifugal and positive rotary pumps, Number 02-06, are amended as set forth below.

Re-write section C.1.7 of MATERIALS to read as follows:

C.1.7.2 Carbon and ceramic materials shall be inert, non-porous, non-toxic, non-absorbent, insoluble, resistant to scratching, scoring, and distortion when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment.

Note: This Amendment will be included in the reprint, Number 02-08.