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**ESCHERICHIA COLI ISOLATED FROM CALVES WITH DIARRHŒA: MANNOSE-RESISTANT HAEMAGGLUTINATION AND COLONISATION FACTOR**

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**Résumé**

ESCHERICHIA COLI ISOLÉ DES VEAUX DIARRHÉIQUES: DÉTERMINATION DE L’ANTIGÈNE K99 PAR DIFFÉRENTES MÉTHODES. — Une étude a été entreprise pour vérifier la validité des méthodes et mettre en évidence la présence du caractère K99 dans les souches de Escherichia coli isolées des veaux diarrhéiques. La détection de l’antigène K99, en 84 souches de Escherichia coli, a été effectuée par le Brush-Border, l’hémagglutination avec et sans mannose et par l’agglutination sur lame. Le pourcentage le plus élevé de positivité a été obtenu par l’agglutination, tandis que les résultats obtenus par le Brush-Border et l’hémagglutination sont inférieurs et méritent des études ultérieures.

The piliated structures, denominated K99, in enterotoxigenic Escherichia coli isolated from calves with diarrhea were described (Ørskov et al., 1975; Burrows et al., 1976). Such a condition also appears in other animal species: pigs (Jones and Rutter, 1972) and lambs (Smith and Linggood, 1972). This antigen is plasmid-mediated (Ørskov and Ørskov, 1966; Ørskov et al., 1975) and has the important characteristic of virulence in its ability to proliferate in the small intestine of young animals (Smith and Linggood, 1972). This kind of activity has also been demonstrated in vitro (Burrows et al., 1976). Escherichia coli carrying the adhesive antigen can be identified by slide agglutination test with specific antisera (Burrows et al., 1976; Guinée et al., 1976). Another common property of these bacteria is the ability to evoke mannose-resistant haemagglutination (Duguid et al., 1955; Burrows et al., 1976).

The purpose of this study is to investigate haemagglutination activity and its significance in K99 positive strains of Escherichia coli detected by the slide agglutination and Brush-Border tests.

**Materials and Methods**

**Bacterial strains**

Eighty-four Escherichia coli strains were isolated from newborn calves with enteric syndrome (Valente et al., 1982). The fecal specimens or intestinal contents were cultured on sheep blood agar and McConkey medium. From each calf one or two representative colonies suspected of being Escherichia coli were chosen for further examination. Escherichia coli were maintained on Trypticase Soy Agar at + 4° C and subcultured every three months. (Escherichia coli B41 (O101 : K99) was used as positive control (Burrows et al., 1976).

**Slide agglutination test**

Escherichia coli B41 was used for the preparation of specific antiserum against K99 antigen. For antiserum preparation, strain carrying the K99 antigen, was cultivated on Minca-Isovitalex medium (Guinée et al., 1977) and inoculated in adult rabbits (Sojka, 1965; Valente et al., 1982). The antiserum was absorbed (Sojka, 1965). The agglutination test was carried out to determine the agglutination titre (table 1).

**Brush-Border test**

Brush-Border test was prepared from the small intestine of newborn calves (Sellwood et al., 1975; Girardeau, 1980). To 100 μl of a suspension of epithelial cells with 2 x 10⁶ cells/ml, was added 100 μl of a suspension of 5 x 10⁹ bacte-
ria/ml in small vials. After mixing slowly for 30 min at room-temperature, the suspension was viewed by phase contrast microscopy (Sellwood et al., 1975). Adhesion index for each strain was evaluated counting the bacteria attached to 20 enterocytes. When the average number of bacteria/cells was \( \geq 10 \) the test was considered positive (Sellwood et al., 1975; Girardeau, 1980). The anti-K99 antiserum (100 \( \mu l \)) was added to the suspension of Brush-Border cells (100 \( \mu l \)) to verify the attachment specificity (Sellwood et al., 1975); the non-specific anti-serum was used too.

**Haemagglutination (HA) and mannose-resistant haemagglutination (MRHA) tests**

*Escherichia coli* strains tested for HA and MRHA were grown on Minca-Isovitalex medium. The bacteria were collected with saline solution (5 \( \times 10^{10} \) colony-forming units/ml) (Jones and Rutter, 1974). Doubled dilution of the bacterial suspension from Minca Isovitalex medium was made with saline solution with and without the addition of 1% D-mannose in Microtiter trays (Jones and Rutter, 1974; Oliveira et al., 1981). Equal volume (25 \( \mu l \)) of red blood cell (rbc) suspension was added. The highest dilution of bacterial suspension giving complete haemagglutination was taken as a final titre, and reactions with titres higher than 1:4 were considered positive. The sheep erythrocytes were obtained fresh and washed four times in saline solution; the packed cells resuspended in phosphate-buffered saline, pH 7.2, at a concentration of 5 \( \times 10^8 \) rbc/ml, were used (Jones and Rutter, 1974).

**Results**

The anti-K99 antiserum absorbed gave an agglutination titer of 1:1280 with the strain *Escherichia coli* B41. The Brush-Border test gave a high index of positivity, with the strain of reference, and the attachment was inhibited by anti-K99 antiserum; the non-specific antiserum did not change in the attachment to the epithelial cells. The haemagglutination with sheep erythrocytes in presence of D-mannose was also positive.

*Escherichia coli* B41 grown at 18°C, at which temperature the K99 antigen is not expressed (Ørskov et al., 1975), in the sero-agglutination and attachment test, and haemagglutination with mannose gave negative result (table 1).

Forty (47.6%) of *Escherichia coli* strains were positive in the slide agglutination test in presence of anti-K99 antiserum (table 2), and 30 of these were positive in the other tests too; whereas six strains were not MRHA and four strains gave positive results only for the agglutination.

The strains with adhesive properties were 47 (55.9%) and of these only 36 were inhibited in the presence of anti-K99 antiserum. The attachment was inhibited by anti-K99 antiserum at the dilution of 1:80.

Forty-one (48.8%) strains were haemagglutinated and only 30 were resistant in the same reaction with D-mannose.

Among the 44 strains negative to slide agglutination test, 28 strains, at the same time, gave neither the attachment nor the haemagglutination with and without D-mannose; while 11 of these strains showed adhesive properties and five strains gave positive results for haemagglutination.

**Discussion**

The highest percentage of positivity, in the

| Table 1. — *Escherichia coli* B41: results of agglutination, Brush-Border and haemagglutination tests. |
|---------------------------------------------------------------|-----------|-----------|
| **Escherichia coli** B41                                      | grown at 37°C | grown at 18°C |
| Slide agglutination test                                      | +         | −         |
| with anti-K99 antiserum absorbed                               |           |           |
| Tube agglutination test                                       | 1 280\( ^a \) | 20        |
| with anti-K99 antiserum absorbed                               | 2 560     | 640       |
| non absorbed                                                  |           |           |
| Brush-Border test                                              | +         | −         |
| inhibition by antiserum K99 antiserum                         | +         | −         |
| Haemagglutination                                             | +         | −         |
| with mannose                                                  |           |           |
| \( ^a \): agglutination titre                                |           |           |
detection of K99 antigen of *Escherichia coli* strains isolated from calves, was obtained by the slide agglutination test, while the results were lower with Brush-Border and MRHA tests.

All three tests were positive contemporaneously only in 30 strains of *Escherichia coli*.

The sero-agglutination test, adhesive factor and haemagglutination activity with D-mannose were not exhibited by K99 positive strain *Escherichia coli* B41 grown at 18 °C, this suggests that the K99 antigen is responsible for all three properties.

The high number of positive strains in the attachment test was reduced when the adhesive test, in presence of anti-K99 immune serum, was performed; in this survey 11 *Escherichia coli* strains were not inhibited.

However, it was necessary to confirm the Brush-Border test specificity by anti-K99 immune serum inhibition (Girardeau, 1980). This suggested that there were present peripherically to bacteria other structures, which were not antigenically similar to K99, but they were equal in their attachment abilities.

The same behaviour was shown in the bacteria isolated from swine; the piliated structures, different from K88 antigen, sometimes might be detected (Jones and Rutter, 1974; Oliveira et al., 1981). These results were not surprising if we consider that the adhesive properties in *Streptococcus pyogenes* (Ellen and Gibbons, 1972) and *Vibrio cholerae* (Freter, 1969) have already been described. The haemagglutinating strains were 41, but when D-mannose was added, the number of positive strains decreased to 30. Therefore this test could also detect structures responsible for the haemagglutination, different from to K99 antigen. The HA gave almost always a titre higher than the MRHA test, however these results remained positive and included in 1:4, 1:8 dilution values.

The percentage of positive results, to detect the K99 antigen, in the MRHA test with sheep erythrocytes was low. In similar studies, the MRHA test with sheep rbc gave reliable results, while the values obtained with the rbc of other animal species were variable (Burrow et al., 1976). Colonisation factors manifested by MRHA of *Escherichia coli*, isolated from children, were almost uniform in some strains and occasional in other strains (Back et al., 1980).

The adhesive and haemagglutinating properties were not the exclusive requisites of the enteropathogenic strains of *Escherichia coli*, but these properties have been recognized in many species of *Enterobacteriaceae*, in which the K99 antigen was not present (Duguid et al., 1955). Attachment is accepted generally as a virulence determinant in the pathogenesis of calf diarrhea.

The diagnostic significance of *Escherichia coli* strains, that exhibit only some characteristics of virulence was not clearly defined (Contrepois et al., 1979); this problem requires further study.

**Table 2.** — Relationship among slide agglutination, Brush-Border and haemagglutination tests in *Escherichia coli* isolated from calves.

<table>
<thead>
<tr>
<th>no. of strains (%)</th>
<th>Slide agglutination test</th>
<th>Brush-Border test</th>
<th>Brush-Border test inhibition by anti-K99 antiserum</th>
<th>Haemagglutination</th>
<th>Haemagglutination with mannose</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 (35.7)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6 (7.1)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>4 (4.7)</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>11 (13.1)</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5 (5.9)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>28 (33.3)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>84</td>
<td>40 (47.6 %)</td>
<td>47 (55.9 %)</td>
<td>36 (42.8 %)</td>
<td>41 (48.8 %)</td>
<td>30 (35.7 %)</td>
</tr>
</tbody>
</table>
The K99 antigen of Escherichia coli strains isolated from calves resembles the K88 antigen of pig enteropathogenic Escherichia coli (Jones and Rutter, 1974) in its ability to attach to enterocytes, and in its haemagglutination and slide agglutination tests, but these characteristics were not always, present at the same time.

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Summary

A study was carried out for detection of the adhesive antigen K99 on 84 Escherichia coli strains isolated from calves with diarrhea. The methods utilized were the Brush-Border and the slide agglutination tests; the strains were also tested in their ability to evoke mannose-resistant haemagglutination. The positive results obtained with the slide agglutination test were higher with comparison to the positive results obtained with other tests. The bacteria had the other piliated structures, besides the K99 antigen, that require further studies.

References


