Evaluation of Transport Media for Pasteurella multocida Isolates from Rabbit Nasal Specimens

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Received 7 October 1996/Returned for modification 7 January 1997/Accepted 30 April 1997

A suitable medium for the transport of Pasteurella multocida in nasal specimens from rabbits was investigated by using pure cultures of the organism and nasal swabs from infected rabbits. First, the ability of eight transport media to preserve the viabilities of P. multocida strains isolated from rabbits was studied. Cary-Blair medium and Leibovitz medium no. 15 (L-15) were found to be superior to the other six media tested, enabling survival of the organism for more than 14 days at room temperature. Second, the survival of P. multocida in nasal specimens was evaluated on both Cary-Blair medium and L-15. The recovery rate of the organism from these two media was more than 80 to 90% during 4 days of storage and decreased gradually with increasing preservation time. There were no significant differences (P > 0.05) in recovery rates of the organism between Cary-Blair medium and L-15. On the basis of these results, we recommend the use of Cary-Blair medium for the transport of P. multocida in rabbit nasal specimens because of the ease of transport of nasal swabs by mail.

Pasteurellosis, caused by Pasteurella multocida, is still the most prevalent bacterial disease in rabbits used for biomedical research. To control the disease, many attempts have been made to develop diagnostic methods for pasteurellosis. In general, diagnosis of the disease is based on the observation of clinical signs, coupled with isolation and identification of the organism (13). Isolation of the organism has commonly been achieved by culture of nasal swab specimens. Immediate culture of nasal swab specimens, however, is not always possible. Accordingly, the use of a proper method for transporting specimens containing P. multocida is necessary for the diagnosis of rabbit pasteurellosis.

Microbiological specimens are transported to the laboratory by various means (7). Many media have been used for this purpose. A buffer type of transport medium such as that of Stuart is often used. Ordinary nutrient broth or anaerobic broth may be used. Recently, swab transport systems such as Culturette (12), Port-A-Cul (10), and Transwab (3) have become commercially available. In the case of rabbit pasteurellosis, a proper transport medium for isolating P. multocida from the nasal cavities of rabbits has not been established. Shimoda et al. (19) showed that it is difficult to maintain a strain of P. multocida isolated from rabbits in phosphate-buffered saline (PBS) and in PBS containing various agents at 37, 24, and 4°C.

The purpose of the present study was to find a suitable medium for the transport of P. multocida in nasal specimens from rabbits.

MATERIALS AND METHODS

Transport media. (i) Eight transport media were used in a comparison of the survival rates of P. multocida strains. They included Cary-Blair medium (2), Stuart medium (20), Schaedler broth (18), thioglycolate broth (without indicator) (1), thioglycolate broth with 0.5% agar, Leibovitz medium no. 15 (L-15) (5), Culturette (Marion Scientific Corp., Rockford, Ill.), and Transwab (Medical Wire and Equipment Company, Corsham, England). (ii) The survival of P. multocida in nasal specimens was compared on Cary-Blair medium and L-15.

P. multocida strains. Sixteen strains of P. multocida maintained in our laboratory were used. All the strains were obtained from the nasal cavities of rabbits with or without rhinitis in four different rabbit colonies in Japan. The identification of P. multocida was performed by procedures described previously (6). These strains had a somatic antigen that had a major reaction with type 12 antiserum by the gel diffusion precipitin test (6). This antigen is the major somatic antigen of P. multocida in the United States (4, 11, 16), Canada (15), and Japan (9). The strains, which were passaged fewer than three times, were stored at −80°C in Trypto-soy broth (Eiken Chemical Co., Ltd., Tokyo, Japan) for 1 to 3 years.

Preparation of P. multocida suspensions. The strain to be tested was grown in Trypto-soy broth at 37°C for 18 h. The suspensions of each culture contained approximately 10^9 CFU/ml. They were diluted with the same broth to give a final concentration of 4 × 10^2 to 7 × 10^5 or 5 × 10^2 to 8 × 10^3 CFU/ml. The numbers of CFU were determined after culturing 0.1 ml of each of the respective suspensions on duplicate plates of 5% horse blood agar.

Inoculation and storage of swabs. Aliquots of 1 ml of the bacterial suspension were transferred to sterile glass tubes (12 by 100 mm). One sterile cotton swab (1 by 150 mm; Eiken Chemical Co., Ltd.) was immersed in each tube for 5 s. Then, each swab was placed into each of the eight transport media described above. Four swabs were used for each medium tested. These were stored at room temperature for different periods of time.

Recovery of P. multocida. After storage, each swab was removed from the transport medium and was streaked onto a blood agar plate. The plate was incubated at 37°C, and the colony formation of the bacteria on the plate was observed at 24 and 48 h.

Viable count determinations. To determine the numbers of P. multocida during storage in Cary-Blair medium, Stuart medium, Schaedler broth, and thioglycolate broth, colony counts were determined on blood agar plates. Each of three P. multocida strains was grown in 30 ml of Trypto-soy broth at 37°C for 18 h. The bacterial cells were collected by centrifugation, washed twice with PBS (pH 7.2), and suspended in 3 ml of PBS. The swabs were immersed in the suspension for 5 s, and then placed into each transport medium. Four swabs were used for each medium tested. The media were stored at room temperature for different periods of time (0, 1, 3, and 5 days). After storage, the swabs were transferred to a tube containing 1 ml of Trypto-soy broth, and the tube was vigorously shaken for 5 s. Serial 10-fold dilutions were made with the same broth, and then 0.1 ml of each dilution was dropped onto one of two blood agar plates. The average numbers of CFU per milliliter in the suspensions were determined as described previously (8).

Rabbits. The study with the rabbit nasal specimens was conducted at five research facilities (facilities A, B, C, D, and E). The nasal swab from each rabbit was cultured for P. multocida. Specimens from 71 rabbits with positive culture results were used in this study.

Specimen collection and handling. Nasal swabs were collected from 20 rabbits in facilities C and E by inserting a sterile cotton swab (1 by 150 mm; Eiken
Chemical Co., Ltd.) into the nasal cavity to a depth of 1.0 to 1.5 cm. Three swabs were obtained from each rabbit. The swabs were each placed in a separate test tube (12 by 100 mm) containing 1 ml of L-15 and transported to our laboratory at room temperature within 1 h. On receipt, one swab was immediately streaked onto kanamycin-bacitracin agar medium (8) to confirm the presence of \( P.\) \( \text{multocida} \). The collected material was eluted in L-15 from the other two swabs by shaking for 5 s. The swab was removed from the tube. The material in one tube was stored at room temperature, and culture of the material was done to determine survival of \( P.\) \( \text{multocida} \) for five different periods of time by using swabs. The new five swabs were immersed in another tube, for 5 s each, placed in Cary-Blair medium, and stored and cultured as described above.

Additional nasal swabs from 51 rabbits at five facilities were taken three or five times. The swabs were immediately placed in Cary-Blair medium. The medium was transported, stored, and cultured as described above.

**Determination of survival of** \( P.\) \( \text{multocida} \) **in infected nasal specimens maintained in** Cary-Blair **medium and L-15.** After storage of the specimens in the media, each swab was removed from the media and inoculated onto kanamycin-bacitracin agar medium (8). The agar plate was incubated at 37°C for 24 and 48 h. The survival of \( P.\) \( \text{multocida} \) was confirmed after identification of the organisms as described previously (8).

**Enumeration of** \( P.\) \( \text{multocida} \) **in nasal specimens.** Concurrent with the survival test, enumeration of \( P.\) \( \text{multocida} \) in 16 nasal specimens from two of five facilities (facilities D and E) was done as described previously (8).

**Statistical analysis.** Comparison of the distribution of viable and nonviable strains from eight transport media at room temperature determined with a bacterial suspension of \( 10^6 \) CFU/ml described above. Cary-Blair medium and L-15 permitted the recovery of all 10 strains for more than 14 days. In contrast, the survival of the organism from thioglycolate was shorter, because no growth of the organism was observed at as early as 4 days or at 14 days.

The results of the other experiment with bacterial suspensions of \( 10^6 \) CFU/ml prepared from 10 of 16 strains (Table 2) were essentially similar to those obtained with bacterial suspensions of \( 10^5 \) CFU/ml described above. Cary-Blair medium and L-15 permitted the recovery of all 10 strains for more than 14 days. Data indicate the average numbers of CFU per milliliter for three strains.

The results of the another experiment with bacterial suspensions of \( 10^6 \) CFU/ml prepared from 10 of 16 strains (Table 2) were essentially similar to those obtained with bacterial suspensions of \( 10^5 \) CFU/ml described above. Cary-Blair medium and L-15 permitted the recovery of all 10 strains for more than 14 days. In contrast, the survival of the organism from thioglycolate, Transwab, Schaedler broth, Culturette, and Stuart medium was markedly shorter. The bacteria in these media could not survive for 1 day.

To confirm the results described above, further investigation was done to determine the numbers of viable \( P.\) \( \text{multocida} \) during storage in Cary-Blair medium, Stuart medium, thioglycolate broth, and Schaedler broth (Fig. 1). The best survival of the organism was obtained on Cary-Blair medium. Although the numbers of viable organisms in Cary-Blair medium decreased by approximately 1 log unit in the first day, the numbers were maintained at the same level throughout the next 4 days. The numbers of CFU of \( P.\) \( \text{multocida} \) stored in Stuart medium gradually decreased with increasing storage time. The organism was not viable in thioglycolate broth and Schaedler broth after 3 days and 1 day, respectively.

**Recovery of** \( P.\) \( \text{multocida} \) **from pure culture in eight transport media.** Table 1 presents the survival rates of \( P.\) \( \text{multocida} \) on the swabs immersed in the bacterial suspensions containing \( 10^6 \) CFU/ml and stored in eight transport media at room temperature. Of the media, Cary-Blair medium and L-15 were the best for survival of the organisms. All the strains inoculated in both media were recovered for more than 14 days. After 14 days, 9 of 16 strains were recovered from thioglycolate with 0.5% agar. For the other five media survival of the organism was shorter, because no growth of the organism was observed at as early as 4 days or at 14 days.

**Recovery of** \( P.\) \( \text{multocida} \) **from rabbit nasal specimens in Cary-Blair medium and L-15.** Since the best survival was shown on Cary-Blair medium and L-15 in the experiments described above, the survival of \( P.\) \( \text{multocida} \) in both media was.

### Table 1. Recovery of 16 \( P.\) \( \text{multocida} \) strains from eight transport media at room temperature determined with a bacterial suspension of \( 10^6 \) CFU/ml

<table>
<thead>
<tr>
<th>Medium</th>
<th>No. of surviving strains on the following days after storage in the media:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Thioglycolate</td>
<td>14</td>
</tr>
<tr>
<td>Transwab</td>
<td>14</td>
</tr>
<tr>
<td>Schaedler</td>
<td>7</td>
</tr>
<tr>
<td>Culturette</td>
<td>16</td>
</tr>
<tr>
<td>Stuart</td>
<td>16</td>
</tr>
<tr>
<td>Thioglycolate with 0.5% agar</td>
<td>16</td>
</tr>
<tr>
<td>Cary-Blair</td>
<td>16</td>
</tr>
<tr>
<td>L-15</td>
<td>16</td>
</tr>
</tbody>
</table>

**RESULTS**

**Recovery of** \( P.\) \( \text{multocida} \) **from pure culture in eight transport media.** Table 1 presents the survival rates of \( P.\) \( \text{multocida} \) on the swabs immersed in the bacterial suspensions containing \( 10^6 \) CFU/ml and stored in eight transport media at room temperature. Of the media, Cary-Blair medium and L-15 were the best for survival of the organisms. All the strains inoculated in both media were recovered for more than 14 days.

**Recovery of** \( P.\) \( \text{multocida} \) **from rabbit nasal specimens in Cary-Blair medium and L-15.** Since the best survival was shown on Cary-Blair medium and L-15 in the experiments described above, the survival of \( P.\) \( \text{multocida} \) in both media was.

### Table 2. Recovery of 10 \( P.\) \( \text{multocida} \) strains from eight transport media at room temperature determined with a bacterial suspension of \( 10^6 \) CFU/ml

<table>
<thead>
<tr>
<th>Medium</th>
<th>No. of surviving strains on the following days after storage in the media:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Thioglycolate</td>
<td>0</td>
</tr>
<tr>
<td>Transwab</td>
<td>0</td>
</tr>
<tr>
<td>Schaedler</td>
<td>0</td>
</tr>
<tr>
<td>Culturette</td>
<td>0</td>
</tr>
<tr>
<td>Stuart</td>
<td>0</td>
</tr>
<tr>
<td>Thioglycolate with 0.5% agar</td>
<td>9</td>
</tr>
<tr>
<td>Cary-Blair</td>
<td>10</td>
</tr>
<tr>
<td>L-15</td>
<td>10</td>
</tr>
</tbody>
</table>

### Table 3. Survival of \( P.\) \( \text{multocida} \) in rabbit nasal samples stored in Cary-Blair medium and L-15 medium at room temperature

<table>
<thead>
<tr>
<th>Medium</th>
<th>No. (%) of samples containing viable ( P.) ( \text{multocida} ) after storage for the following periods (days):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cary-Blair</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>18 (90)</td>
</tr>
<tr>
<td>L-15</td>
<td>20 (100)</td>
</tr>
</tbody>
</table>

*There was no statistically significant difference between the two media in the recovery rate of the organism.*
compared by using nasal specimens from facilities C and E (Table 3). The recovery rate of the organisms in both media was more than 80% (16 of 20) to 90% (18 of 20) after 4 days and then decreased gradually. There were no significant differences (P > 0.05) in the recovery rates of the organism between the two media.

The survival of *P. multocida* in nasal specimens in Cary-Blair medium was further examined (Table 4). When the nasal swabs obtained from 51 infected rabbits were placed directly in Cary-Blair medium and stored, the recovery rates of the organism from the medium were slightly higher than those listed in Table 3. An influence of the sources of the specimens on the recovery rate was observed. At facility D, the survival of *P. multocida* was unstable. The mean numbers of viable *P. multocida* in nasal swabs from facilities D and E were 10^4.9 and 10^5.3 CFU/ml, respectively.

**DISCUSSION**

Our present study showed that Cary-Blair medium and L-15 are superior to the other media tested with regard to the survival of *P. multocida* in pure cultures and nasal specimens from infected rabbits.

Various types of media such as buffer types of transport media and broth media have been used to transport bacterial specimens. In addition, several commercially available transport systems have been developed (3, 10, 12). The most popular transport medium for general-purpose use is the buffer type of semisolid medium containing a reducing agent and limiting nutrients, such as Stuart medium (14). Ordinary nutrient broth or anaerobic broth have also been used (7). However, little work on evaluations of transport media for the isolation of *P. multocida* from nasal specimen from rabbits has been published. Shimoda et al. (19) reported that the survival of *P. multocida* strain from a rabbit was poor in PBS at 37, 24, and −20°C. The poor survival was, however, improved by adding skim milk and glucose to the PBS and keeping it at −20°C, but not at 4, 24, or 37°C. Also, little work has been done on transporting *P. multocida* specimens from domestic animals other than rabbits. Chanter et al. (3) reported the effects of storage in a transport medium (Transwab) and storage temperature on toxigenic *P. multocida* from the nasal cavities of pigs. They showed that the recovery rate of the organism from nasal specimens from infected pigs decreased to 50% after 48 h when the specimens were kept in the medium at 10°C. It was therefore important to find the best medium for transporting specimens containing *P. multocida*.

Our data with *P. multocida* strains indicate that the stability of the strains at room temperature was greater in Cary-Blair medium and L-15 among the eight transport media tested. The recovery rate of the organism from nasal specimens from infected rabbits for specimens kept in both media at the same temperature was more than 80 to 90% after storage. The results demonstrate that *P. multocida*-containing specimens from the nasal cavities of rabbits can be successfully kept in Cary-Blair medium and L-15 for at least 4 days at room temperature. It is good that the method allows specimens to be transported at room temperature, so that they do not need to be cooled or frozen. In Japan, microbiological specimens can arrive at the laboratory within about 2 days by mail.

We may propose that Cary-Blair medium be used to transport *P. multocida* in rabbit nasal specimens because of the ease of transport of nasal swabs by mail. This medium is recommended for enteric pathogens, such as *Vibrio parahaemolyticus* and *Campylobacter jejuni* (17), while Stuart medium has been widely used to transport various specimens other than fecal specimens. In our study, Cary-Blair medium was superior to Stuart medium for transporting *P. multocida*-containing nasal specimens from rabbit. The results suggest that Cary-Blair medium may be used to transport *Pasteurella* specimens from other domestic animals and humans. Percy et al. (15) used Cary-Blair medium as the transport medium for a survey of serotypes of *P. multocida* from rabbits in Canada. Also, these data suggest that the medium may be used to transport pathogens other than enteric pathogens, such as respiratory pathogens.

In conclusion, Cary-Blair medium is preferred for the transport of *P. multocida* specimens. L-15 may be useful in laboratory as a routine diluent for *P. multocida*. Further work, however, is needed to investigate the mechanism of survival of *P. multocida* isolates kept in both media.

**ACKNOWLEDGMENTS**

We are grateful to the staff of research facilities for their assistance in collecting samples. We also thank E. Okiyama for excellent technical assistance.

This study was partly supported by a Grant-in-Aid for Scientific Research (A) (no. 08308041) from the Ministry of Education, Science, Sports and Culture, Japan.

**REFERENCES**