Comparison of Four Culture Media for Isolation of *Mycobacterium avium* Complex from Porcine Tissues

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The efficiency of four culture media was compared for the isolation of *Mycobacterium avium* complex from 197 porcine tissues. In 82 tissues with microscopic granulomas and acid-fast bacilli, a significantly greater number of isolates were obtained on Middlebrook 7H10 medium with sodium pyruvate than on Stonebrink medium, Herrold egg yolk agar medium, or Lowenstein-Jensen medium ($P = 0.01$). In 46 tissues in which no microscopic granulomas or acid-fast bacilli were observed, a significantly greater number of isolates were made on Middlebrook 7H10 medium or Herrold egg yolk agar medium than on Stonebrink medium or on Lowenstein-Jensen medium ($P = 0.01$). The time required to grow *M. avium* complex on Lowenstein-Jensen medium was significantly greater than the time required to observe growth on Stonebrink, Middlebrook 7H10, or Herrold egg yolk agar medium ($P = 0.001$).

Mycobacterium avium complex is considered the most common etiological agent of swine tuberculosis in the United States (10, 11). Although reports are available comparing various media for the isolation of *M. tuberculosis* (2, 3), no specific information is available on the efficiency of culture media for isolating *M. avium* complex. The purpose of this report is to present information on the isolation of *M. avium* complex from 197 porcine lymph nodes which were positive upon bacteriological examination.

MATERIALS AND METHODS

The following culture media with certain modifications were prepared as previously described (13): Lowenstein-Jensen (Difco Laboratories, Detroit, Mich.) with glycerol, 12 ml/1.6 liter (LJ); Stonebrink (S); Middlebrook 7H10 (Difco) with sodium pyruvate, 4.1 g/liter (M); and Herrold egg yolk agar media with mycobactin, 2 mg/liter, and malachite green (H).

A total of 197 lymph node specimens from swine suspected of being tuberculous were included in the present study. When collected at the abattoir, each lymph node was divided equally; one part was placed in saturated sodium borate solution, and the other part was placed in 10% Formalin (except in 15 cases, no tissues were received in Formalin).

Tissues for bacteriological examination were rinsed in a dilute solution of sodium hypochlorite (1:1,000) as soon as possible after receipt in the laboratory. All fat was removed from tissues by using separate sterile

scissors and forceps for each specimen. Specimens were placed in fresh hypochlorite solution (1:1,000) and were stored overnight at 4°C.

The tissues were rinsed in fresh hypochlorite and transferred to a blender jar along with 50 ml of nutrient broth as previously described and blended for 2 min (10). Four tubes of culture media (one each of LJ, S, M, and H) were inoculated at random with the tissue suspension by using cotton-tipped applicators. The inoculated slants were observed for growth at 7-day intervals for 8 weeks.

Smears of colonies were stained with carbol-fuchsin. Acid-fast isolants were characterized by morphological and biochemical tests (13); *M. avium* complex isolates were identified by drug susceptibility, biochemical tests, and agglutination test (7, 9, 11).

Paraffin sections of Formalin-fixed tissue of 182 lymph nodes were stained with hematoxylin and eosin and with Auramine-O for the detection of acid-fast bacilli (5).

Tissue sections with granulomas in which acid-fast bacilli could be demonstrated were classified as compatible for mycobacterioses; tissues in which granulomas were present but in which no acid-fast bacilli could be detected were classified as suggestive for mycobacterioses. Tissues in which neither granulomatous lesions nor acid-fast bacilli could be found were classified as negative for mycobacterioses.

Statistical analyses comparing the frequency of isolation of *M. avium* complex on different media for the various histopathological classes were made by analysis of variance. Paired *t* tests were made to compare the time required for growth of *M. avium* complex; this included only those cases on which positive cultures were obtained on the two media being compared.

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RESULTS

The frequency of *M. avium* complex isolations on four culture media is shown in Table 1. In 82 tissues with microscopic granulomas and acid-fast bacilli, a significantly greater number of isolates were obtained on M medium than on S, H, or LJ media ($P = 0.01$). In 46 tissues in which no microscopic granulomas or acid-fast bacilli were observed, a significantly greater number of isolates were made on M medium and on H medium than on S medium or LJ medium ($P = 0.01$). No significant differences were detected in a number of isolations obtained on the four media in 54 cases with microscopic granulomas but no acid-fast bacilli ($P > 0.1$). The frequency of isolation on 15 cases in which no histopathological examinations were conducted was not significantly different ($P > 0.1$).

The time required to grow *M. avium* complex on each of the four media is shown in Table 2. The time required to observe growth of *M. avium* complex on LJ medium was significantly greater than the time required to observe growth on S, M, and H media ($P = 0.001$). The time required for observing growth of *M. avium* complex on H medium was significantly greater than for M medium ($P = 0.01$). No significant differences were observed when comparisons were made for other pairs of culture media ($P > 0.1$).

The number of isolations of serotypes of *M. avium* complex obtained from the 197 porcine lymph nodes is shown in Table 3. No consistent differences could be detected in kinds of serotypes from tissues with either granulomatous lesions with acid-fast bacilli (compatible) or granulomatous lesions with no acid-fast bacilli (suggestive). Serotypes 1, 2, and 10 accounted for 71% of the isolations. Greater numbers of *M. avium* complex serotypes 1 and 2 were isolated from compatible and suggestive lesions, whereas serotype 10 accounted for the greatest number from tissues in which no microscopic lesions were observed.

TABLE 1. Frequency of *M. avium* complex isolations on four culture media

Histopathological class ^a	No. of Cases	No. of isolations by culture media			
		S	LJ	M	H
Compatible	82	51	41	61	49
Suggestive	54	38	38	42	40
Negative	46	18	17	28	38
No exam	15	8	7	9	7

^a Compatible = microscopic granuloma with acid-fast bacilli observed; suggestive = microscopic granuloma with no acid-fast bacilli observed; negative = no microscopic granuloma or acid-fast bacilli observed; no exam = tissue not available for examination.

TABLE 2. Mean number of days for isolation of *M. avium* complex on four culture media

Media comparison	No. of cases	Mean	Days ^a	Mean difference	Computed <i>t</i>
S vs LJ	72	27	30	3	4.2
M vs LJ	78	26	30	4	4.1
H vs LJ	80	28	30	2	3.4
S vs H	84	28	29	1	1.3
M vs H	96	26	29	3	2.8
M vs S	88	27	28	1	1.1

^a Includes only those cases in which *M. avium* complex grew on both media compared.

TABLE 3. Number of serotypes of *M. avium* complex isolated for each of three histopathological classes^a

<i>M. avium</i> complex serotype	Total no.	Compatible	Suggestive	Negative	No microscopic examination
1	32	16	13	3	5
2	66	36	19	11	1
4	15	7	4	4	0
8	17	5	4	8	0
10	30	8	9	13	2
Other ^b	10	4	2	4	6
Mixed or "double" types ^c	9	5	3	1	1
Untypable	3	1	0	2	0

^a Histopathological classes: Compatible = granulomas with acid-fast bacilli; suggestive = granulomas with no acid-fast bacilli observed; negative = no granulomas or acid-fast bacilli found.

^b Includes one or more isolates of *M. avium* complex serotypes 5, 7, 9, 11, 12, 16, and 20.

^c Includes isolates which share antigens with two or more recognized serotypes.

DISCUSSION

The finding that M medium accounted for a significantly greater number of isolations is of practical and economic importance. Isolates made on this media are usually smooth and readily serotyped after a single subculture in either liquid or solid media, thereby minimizing the need for additional subculture (11, 12). It should also be emphasized that no problems were encountered with contamination on M medium in any of the 197 positive cases. The equipment and personnel available for media preparation is an important consideration in deciding which of the media to use. Middlebrook 7H10 base medium is commercially available and does not require special processing; however, LJ medium requires a suitable egg source and must be inspissated.

It is difficult to determine the significance of data comparing the number of isolations completed with a single tube of each of four media.

However, the finding of an increased number of isolations on H medium with mycobactin suggests that the growth of certain strains of *M. avium* complex may be stimulated by this factor, which has been reported to be a chelating agent (4, 8). It is interesting that serotypes other than 1 and 2 accounted for 70% (32/46) *M. avium* complex isolates from submissions in which no granulomas or acid-fast bacilli were found. This information provides evidence that H medium with mycobactin should be included for the isolation of *M. avium* complex particularly when microscopic examination fails to reveal the presence of acid-fast bacilli in tuberculous lesions of swine.

The relatively poor performance of LJ medium in isolating *M. avium* complex and a greater rate of contamination suggest it may not be useful in routine clinical cases. However, LJ medium is reported to be one of the most suitable media for the isolation of *M. tuberculosis* (2, 3). Because this organism has been isolated from swine (11) there is justification for its use in veterinary mycobacteriology laboratories.

Similarly, the mediocre performance of S medium in isolating *M. avium* complex raises questions concerning its use for routine specimens. This medium, which contains pyruvate, has been recommended for the isolation of *M. bovis* (1, 6). *M. bovis* infection has been reported in swine (10, 11); therefore, this may provide some basis for recommending its continued use. Because different species of the genus *Mycobacterium* have been isolated from porcine tissues, the use of all four mediums for routine bacteriological examination on swine tissues is recommended.

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