

RESEARCH COMMUNICATION

ADDITION OF RABBIT SERUM TO EMJH MEDIUM IMPROVES ISOLATION OF LEPTOSPIRA INTERROGANS SEROVAR HARDJO

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ABSTRACT

TE BRUGGE, LESLEY A. & LOUW, H. NICOLETTE, 1985. Addition of rabbit serum to EMJH medium improves isolation of *Leptospira interrogans* serovar *hardjo*. *Onderstepoort Journal of Veterinary Research*, 52, 53-54 (1985).

The addition of 2 % pooled rabbit serum to semi-solid commercial EMJH medium with EMJH enrichment and 0,5 mg of 5-fluorouracil per ml was found to enhance the growth rate and success of isolation of *Leptospira interrogans* serovar *hardjo* from bovine urine. Cultures made on media without serum had to be kept for more than 130 days before being discarded as negative.

INTRODUCTION

Leptospira interrogans serovar *hardjo* (*L. hardjo*) is known to be a fastidious grower, and until media containing bovine serum albumin (BSA) came into use, few isolations were made (Ellinghausen, 1980). Ellis, O'Brien, Neill, Ferguson & Hanna (1982) showed that the addition of 2 % rabbit serum enhanced the isolation and growth of leptospirae in EMJH medium. Thiermann & Ellinghausen, (1981) proved that where high quality BSA is used, this enhancement is achieved without the addition of serum.

In laboratories where relatively little medium is used, the time-consuming, prior evaluation of consignments of BSA is not justified. This trial was therefore undertaken to see whether or not the addition of 2 % pooled rabbit serum to the routinely used medium improved isolation results.

MATERIAL AND METHODS

Source of specimens

Urine was collected from a dairy herd known to be infected with *L. hardjo* on 2 occasions 14 days apart, and was processed according to the methods of Herr, Riley, Nesor, Roux & De Lange (1982).

Media

The standard medium (Medium A) was semi-solid EMJH (0,15 % agar)¹ with EMJH enrichment with 0,5 mg of 5-fluorouracil² per ml medium. Medium B was the same as Medium A, but with the addition of 2 % pooled rabbit serum. The media were dispensed in 5 ml quantities in screw-capped, tissue culture tubes, screened for contaminants and stored at 4 °C until use. Four tubes of each type of medium were used for each specimen.

RESULTS

The number of isolations (tubes showing growth) per specimen for each type of medium is shown in Table 1.

In the 1st attempt, 5/6 specimens were positive on Medium B (containing rabbit serum) and 2/6 on Medium A. Medium B showed growth on 12 out of a possible 24 tubes (50 %), and Medium A 6 out of 24 tubes (25 %).

In the 2nd attempt, growth was seen in both Media A and B from 5 out of 6 specimens. Medium B showed growth in 15 out of 24 tubes (62,5 %) and A showed growth in 13 out of 24 tubes (54 %).

The time taken for growth to be noted in the culture tubes from both isolation attempts is shown in Table 2.

Nineteen tubes were positive on Medium A, and 28 on Medium B. In the 1st attempt, 11/13 (84,6 %) of the tubes became positive by Day 49 on Medium B, while none were positive at that stage on Medium A. The first to become positive on Medium A did so between Days 50 and 63. The last to become positive on Medium B did so by Day 105, and on Medium A by Day 131.

TABLE 1 Number of tubes showing growth of *Leptospira* for each type of medium out of a possible 4 per specimen per medium

Cow No.	First attempt		Second attempt	
	A	B	A	B
1	—	1	3	3
2	—	1	3	3
3	2	4	1	2
4	—	—	4	3
5	4	3	2	4
6	—	3	—	—
Total	6	12	13	15

A = semi-solid EMJH with EMJH enrichment and 0,5 mg 5-fluorouracil per ml medium

B = as for A, with 2 % pooled rabbit serum

TABLE 2 Time taken for tubes to show growth of *Leptospira*

Medium	Days									Totals
	0-28	29-35	36-42	43-49	50-63	64-77	78-91	92-105	106-131	
A1	—	—	—	—	1	—	2	2	1	6
2	4	1	—	1	1	2	3	—	—	12
B1	—	—	11	—	1	—	—	1	—	13
2	9	2	—	1	3	—	—	—	—	15
Total tubes showing growth	13	3	11	2	6	2	5	3	1	46

A = semi-solid EMJH with EMJH enrichment and 0,5 mg 5-fluorouracil per ml medium

B = as for A, with 2 % pooled rabbit serum

1 = 1st attempt at isolation

2 = 2nd attempt at isolation

¹ Difco Laboratories, Detroit, Michigan USA

² Roche Products (Pty) Ltd, 4 Brewery St., Isando.

In the 2nd attempt, 6 of the 12 positives (50 %) became positive by Day 49 on Medium A, and 12 of the 15 positive (80 %) on Medium B. The last tube on Medium B became positive by Day 63, and on Medium A by Day 91.

DISCUSSION

The addition of 2 % rabbit serum to the medium used for the primary isolation of *L. hardjo* reduced the time required to culture the organisms and increased the number of isolates made. This result agrees with the findings of Ellis *et al.* (1982). It also indicates that the BSA in the medium was not of the best quality (Thiermann & Ellinghausen, 1981). Without the addition of the serum the cultures would have had to be kept for much longer (130 days) before being discarded as negative. This compares reasonably with Ellis *et al.* (1982), that some cultures only became positive after 16 weeks (112 days). Rabbit serum can therefore be used profitably where the time and facilities to evaluate the quality of BSA in the enrichment media are lacking.

The effect of the retarded growth being due to high levels of 5-fluorouracil in the medium is negated by the findings of Johnson & Rogers (1964), who showed that

high levels (up to 1 mg/ml) of 5-fluorouracil did not impede growth, and that early growth occurred on Medium B.

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