

(SHORT COMMUNICATION)

EFFECT OF SOLID MEDIA ON THE GROWTH OF *MYCOPLASMA MYCOIDES* SUBSPECIES *MYCOIDES* IN CONTAGIOUS BOVINE PLEUROPNEUMONIA VACCINE

By

S. A. GARBA

National Veterinary Research Institute,
Vom, Via Jos.

In the preparation of contagious bovine pleuropneumonia (CBPP) Wet T₁ vaccine in tryptose broth the importance of the solid medium lies in the fact that it is used for the determination of the number of viable organisms present in the culture. The number of viable organisms helps to determine the potency of the vaccine and the shelf life (Garba, 1980). Factors affecting the growth of the mycoplasma on the solid medium could therefore lead to erroneous results being obtained during counts of the number of viable organisms.

Bacto PPLO (Difco 1953) has been a favourite solid medium for viable counts of mycoplasmas. Few workers have compared the growth of *M. mycoides* subsp. *mycoides* on different solid media. Menders and Coelho (1962) found that the agar they prepared in their laboratory (glucose/agar) supported the growth of *M. mycoides* better than Bacto PPLO agar.

Cultivation in an atmosphere containing increased concentrations of carbon dioxide may also improve the growth of mycoplasmas on solid media. Stewart and Chowdray (1968) reported that mycoplasmas could be more readily isolated from the human respiratory tract if the cultures were incubated in 10% carbon dioxide atmosphere.

For this investigation the growth of *M. mycoides* subsp. *mycoides* on tryptose agar prepared as described by Brown *et al.* (1965) and modified by the addition of

disodium hydrogen orthophosphate and potassium hydrogen orthophosphate (Karst, O. 1969 unpublished) with the use of bovine serum inactivated at 56°C for 30 minutes and Bacto PPLO agar as formulated in Difco (1953) were compared. One percent Bacto PPLO serum fraction was used as an enrichment in the preparation of the PPLO agar. Before dispensing tryptose agar into plates, 70ml of the following sterile enrichment was added to each 200ml of melted agar:-

Serum (inactivated at 56 ± C for 30 minutes)	60ml
Glucose (10% solution).....	10ml
Yeast extract (10% solution).....	2ml
Sodium penicillin.....	200,000 i.u.
Mycostatin	12,500 i.u.
Sulphadimidine sodium (331/3% solution)	0.4ml

Viable counts of 16 batches of CBPP vaccine were carried out using both tryptose agar and Bacto PPLO agar for each batch of vaccine. In each case the number of viable organisms detectable following growth on tryptose agar was greater than on PPLO agar (Table 1). Thus, while an average of 1.79×10^9 colony forming units per millilitre (cfu/ml) was counted on tryptose agar the corresponding count on tryptose agar the corresponding count on PPLO agar averaged 4.33×10^8 cfu/ml.

TABLE 1

Results of viable counts using Tryptose Agar or Bacto PPLO Agar.

Batch	Tryptose		Agar	PPLO		Agar
1	3.40	×	10 ⁹	8.97	×	10 ⁸
2	2.48	×	10 ⁸	1.16	×	10 ⁸
3	6.50	×	10 ⁹	1.06	×	10 ⁹
4	2.16	×	10 ⁹	8.52	×	10 ⁹
5	9.80	×	10 ⁸	3.53	×	10 ⁸
6	9.56	×	10 ⁸	4.60	×	10 ⁸
7	8.70	×	10 ⁸	2.52	×	10 ⁸
8	1.20	×	10 ⁹	4.22	×	10 ⁸
9	1.40	×	10 ⁸	5.00	×	10 ⁷
10	9.70	×	10 ⁸	2.20	×	10 ⁸
11	2.10	×	10 ⁹	5.77	×	10 ⁸
12	5.20	×	10 ⁹	8.75	×	10 ⁸
13	8.90	×	10 ⁸	1.02	×	10 ⁸
14	1.10	×	10 ⁹	4.98	×	10 ⁸
15	9.90	×	10 ⁸	4.98	×	10 ⁸
16	5.80	×	10 ⁸	9.00	×	10 ⁷

*Colony forming units per ml. All counts by the method of Miles and Misra (1938).

It is not well understood why tryptose agar supported better growth. It may be that because *M. mycoides* subsp. *mycoides* (strain T₁) is routinely propagated on tryptose agar and broths the organisms are more adapted to growing on tryptose agar than ON PPLO agar.

Since tryptose agar is cheaper than the PPLO agar and since it appears to support better growth than PPLO agar, its use for the viable count of *M. mycoides* in T₁ tryptose broth vaccine is recommended.

ACKNOWLEDGEMENT

This paper is published by the permission of the Director, National Veterinary Research Institute, Vom, Nigeria.

BROWN, R.D., GOURLAY, R.N. and McCLEOD, A.K. (1965). The production of T₁ Broth Culture contagious Bovine pleuropneumonia vaccine. *Bull. Epizoot. Dis. Afr.*, 13; 149-155.

DIFCO LABORATORIES (1953). *Difco manual*, Ninth Edition, P. 89-90.

GARBA, S.A. (1980). Shelf life of wet T₁ broth vaccine for contagious bovine pleuropneumonia. *Trop. Anim. Hlth. Prod.* 12; 189-191.

MENDES, A.M. and COELHO, M.A.T. (1962). Notes on the cultivation on solid media of mycoplasma *mycoides*. *Bull. Epizoot. Dis., Afr.*, 10; 59-66.

STEWART, S.M. and CHOWDRAY, J.E. (1968). Isolation of mycoplasmas from human respiratory tract. *J. Path. Bact.*, 95; 580.

MILES, A.A. and MISRA, S.S. (1938). The estimation of the bactericidal power of the blood. *J. Hyg. Camb.* 38; 732.