Failure of Erythritol to Stimulate the Growth of *Salmonella dublin* *in vitro*

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Salmonella dublin is a well-known cause of bovine abortion and was responsible for 309 (2·8%) of 14116 abortions investigated in England and Wales between July 1968 and June 1969 (Hugh-Jones, 1971). The literature has been reviewed (Hinton, 1971). Abortion may be associated with symptoms of dysentery or it may occur in the absence of any other clinical signs. *Salmonella* can usually be isolated from the foetus or the foetal membranes in pure culture, and a serological response can be demonstrated in most cases.

Smith and his colleagues (Smith *et al.* 1962) have shown that the polyol, erythritol, may be one of the main factors responsible for the localization of *Brucella abortus* in the bovine uterus. Erythritol stimulates the growth of *Brucella abortus*, *B. melitensis* and *B. suis* (Williams, Keppie & Smith, 1964). *Brucella abortus* will grow in media in which erythritol is the only carbohydrate (McCullough & Beal, 1951) and the organism will use the polyol in preference to glucose, sorbitol or fructose as a carbon and energy source (Anderson & Smith, 1965). Erythritol is concentrated in the bovine chorion, cotyledons and foetal fluids (Williams, Keppie & Smith, 1962) and it is also found in placental extracts of other hosts susceptible to brucella infections, including sheep, goats and pigs (Smith *et al.* 1962).

This report records results of observations made to determine whether erythritol stimulates the growth of *Salmonella dublin* *in vitro*, as such a finding would indicate possible activity *in vivo*.

METHODS AND RESULTS

The erythritol (meso-erythritol, British Drug Houses Ltd, Poole, Dorset) used in solid medium was sterilized in solution by filtration (Carlson-Ford, Ashton-under-Lyne, Lancashire, grade E K) while the liquid medium was sterilized by tyndallization. All cultures were incubated at 37 °C in air and the concentrations of medium additives are expressed as % (w/v) except where stated.

It was found that field strains of *Brucella abortus* produced acid when seeded on to a medium containing tryptose blood agar base (Difco, Detroit, Michigan, USA), 5% (v/v) horse serum, 1% erythritol and 0·0073% phenol red and incubated in an atmosphere containing 10% (v/v) CO₂. Initial investigations were made to see whether *Salmonella dublin* would metabolize erythritol in a similar manner. However, there was no evidence of acid production when 62 strains of *S. dublin* (39 abortion, 16 faecal, 5 calf and 2 vaccine strains) were grown for 72 h on *Salmonella* Shigella medium CM 99 (Oxoid Ltd, Southwark Bridge Rd, London) containing 1% erythritol. Similarly 19 strains of *S. dublin* (14 abortion, 2 faecal, 2 calf and 1 vaccine strain) failed to produce acid when grown for 7 days in peptone water (Oxoid CM 9) containing 1% (v/v) Andrade's indicator and either 0·125%, 0·25%, 0·5%, 1% or 2% erythritol. Five of the strains in these peptone water cultures were incubated for a further 7 days after the addition of horse serum, again with negative results.

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Finally there was no evidence of oxidation or fermentation when 14 *S. dublin* strains (10 abortion and 4 faecal strains) were grown in the medium of Hugh & Leifson (1953) containing 1% erythritol.

In a more detailed study the growth of 8 abortion strains of *Salmonella dublin* was compared in peptone water alone, and in peptone water containing 1% of either lactose, erythritol or glucose. *Salmonella dublin* organisms, suspended in peptone water, were seeded (0.02 ml) into each of 3 tubes containing 10 ml of the respective media. The initial inoculum for the 8 strains, as judged by surface viable counts on tryptose blood agar base, varied between 3 and 30×10^4 viable organisms. The cultures were incubated for 24 h, killed by autoclaving and the concentration of the bacterial suspension assessed turbidometrically. A polychromatic light source, and the photo-electric cell of a titrator (Evans Electroselenium

Table 1. *Growth of Salmonella dublin in liquid media*

<i>Salmonella dublin</i> strain	Turbidity score†			
	Peptone water	Peptone water containing 1%		
		Lactose	Erythritol	Glucose
1	9.3	7.5	10.5	18.2
2	6.0	6.9	6.6	23.6
3	7.2	7.1	7.3	19.2
4	9.7	15.1	9.8	17.6
5	8.8	8.3	8.1	24.7
6	6.0	6.0	6.1	16.9
7	8.5	15.3	9.1	31.0
8	8.0	6.7	7.3	30.2
Mean value	7.9	9.1	8.1	22.7

† Scores were calculated as described in the text; values for each strain are means of triplicate determinations.

Ltd, Halstead, Essex) were coupled with a galvanometer (Evans Electroselenium Ltd, Unigalvo) and used as a colorimeter. The suspension was placed in an optical cell of 1.0 cm light path, at right angles to the light beam. The instrument was kept in a subdued light and the transmittance value measured on the logarithmic scale of the galvanometer. The turbidity score was obtained by subtracting the reading obtained for the culture in suspension from that for the supernatant fluid after bacteria had been deposited by centrifugation.

The instrument was calibrated with suspensions of known viable count; *Salmonella dublin* was cultured, in either peptone or 1% glucose peptone, and counts were made after 4, 8, 16 and 24 h of incubation. The turbidity score increased from 1 through 5 to 15 as the count rose from 5×10^5 to 1×10^8 to 5×10^8 organisms/ml respectively. With scores between 20 and 45 the counts fell slightly from 4 to 1.5×10^8 organisms/ml indicating that, as the culture was in the stationary or early decline phase, the increased turbidity was due to an accumulation of dead bacteria.

The mean turbidity value for the triplicate determinations was calculated for each strain (Table 1) and showed that, though there was little difference in the turbidity of the cultures in either peptone or the erythritol peptone, there was an increase in turbidity when glucose was added to the basic medium.

The turbidity scores were also measured for 9 strains grown for 1 week in the peptone water containing between 0.125% and 2% erythritol. Although there were some individual variations in the turbidity scores, there was no evidence of an increased turbidity with the increasing concentration of the polyol.

CONCLUSION

The results of both the qualitative and quantitative observations suggest that erythritol neither stimulates nor inhibits the growth of *Salmonella dublin* *in vitro*. Trivett & Meyer (1967) and Lowrie & Pearce (1970) also found that erythritol had no effect on the growth of *Listeria monocytogenes* and *Vibrio fetus* *in vitro*. Taken together these results suggest that, of the bacteria commonly associated with bovine abortion, *Brucella abortus* is probably alone in its ability to metabolize erythritol and that other factors are probably responsible for the localization of *S. dublin* and the other two organisms in the bovine genital tract.

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REFERENCES

- ANDERSON, J. D. & SMITH, H. (1965). The metabolism of erythritol by *Brucella abortus*. *Journal of General Microbiology* **38**, 109-124.
- HINTON, M. (1971). Salmonella abortion in cattle. *Veterinary Bulletin* **41**, 973-980.
- HUGH, R. & LEIFSON, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram negative bacteria. *Journal of Bacteriology* **66**, 24-26.
- HUGH-JONES, M. E. (1971). Bovine abortion and VIDA. *Veterinary Record* **89**, 201-202.
- LOWRIE, D. B. & PEARCE, J. H. (1970). The placental localisation of *Vibrio fetus*. *Journal of Medical Microbiology* **3**, 607-614.
- MCCULLOUGH, N. B. & BEAL, G. A. (1951). The growth and manometric studies on carbohydrate utilisation of *Brucella*. *Journal of Infectious Diseases* **89**, 266-271.
- SMITH, H., WILLIAMS, A. E., PEARCE, J. H., KEPPIE, J., HARRIS-SMITH, P. W., FITZGEORGE, R. B. & WITT, K. (1962). Foetal erythritol. A cause of the localisation of *Brucella abortus* in bovine contagious abortion. *Nature, London* **193**, 47-49.
- TRIVETT, T. L. & MEYER, E. A. (1967). Effect of erythritol on the *in vitro* growth and respiration of *Listeria monocytogenes*. *Journal of Bacteriology* **93**, 1197-1198.
- WILLIAMS, A. E., KEPPIE, J. & SMITH, H. (1962). The chemical basis of the virulence of *Brucella abortus*. III. Foetal erythritol a cause of the localisation of *Brucella abortus* in pregnant cows. *British Journal of Experimental Pathology* **43**, 530-537.
- WILLIAMS, A. E., KEPPIE, J. & SMITH, H. (1964). The relation of erythritol usage to virulence in the brucellas. *Journal of General Microbiology* **37**, 285-292.