

## The Survival of *Mycoplasma suis* in Liquid Medium, on Solid Medium and in Pneumonic Tissue

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**SUMMARY.** The survival time of *Mycoplasma suis* in different circumstances, as judged by the subsequent recovery of the organism in liquid medium, was observed. In liquid medium at room temperature, the mycoplasma survived for 18 to 31 days; and in liquid medium in the refrigerator, it survived for 7 to 14 weeks. The survival time on solid medium incubated at 37°C. was at least 20 days. In small pieces of pneumonic tissue, the survival time was 7 days at room temperature and at least 11 days in the refrigerator.

SOME PIG HERDS which have been clinically and pathologically free from enzootic pneumonia for several years have suddenly developed this disease without the introduction of any live pigs (Goodwin, in preparation). Such apparent reinfections raise questions on the epidemiology of enzootic pneumonia, particularly on the extent to which the causal mycoplasma (*Mycoplasma suis*) may survive outside the pig and be transferred from one herd to another indirectly. The work now reported summarizes experiments on the survival of *M. suis* in liquid medium, on solid medium, and in small pieces of pneumonic tissue.

### MATERIALS AND METHODS

The liquid and solid medium, and the cultural methods, were as previously described (Goodwin & Hurrell, 1970; Goodwin & Pryor, 1970). The starting time for the survival experiments in liquid medium was when freshly-seeded cultures had reached a pH of 6.8 to 6.9; in these experiments, the cultures kept at room temperature were in a dark cupboard. When the survival time in liquid medium and in lung tissue was observed, titrations were made initially, and periodically thereafter, in liquid medium. The solid-medium cultures were incubated at 37°C. In all the experiments, the organism grown at the beginning and at the end was identified as *M. suis* by the metabolic-inhibition test (Taylor-Robinson *et al.*, 1966), except with lung tissue left on the bench. Here the growth obtained after 3 days was identified as *M. suis*, but the growth in liquid medium after 7 days was insufficient to provide satisfactory passages for the

metabolic-inhibition test. However, colonies resembling *M. suis* were obtained on solid medium from the same tissue samples after 7 days on the bench.

The pneumonic tissue, which had been deep frozen, came from experimental and field cases of enzootic pneumonia. Small samples of  $\frac{1}{4}$ – $\frac{1}{2}$  g. were left in covered Petri dishes on the bench and in the refrigerator, the starting point being when the tissue was first thawed.

### RESULTS

#### *Survival in Liquid Medium*

**Room Temperature.** In the first experiments, the temperature varied between 21° and 26.5°C. Four cultures with an initial titre of  $10^{-7}$  or  $10^{-8}$ , declined to  $10^{-5}$  in about 10 days, and to  $10^{-2}$  in about 22 days; when next titrated at 25 days, no growth was obtained. In the next experiments, the temperature varied between 18° and 29°C. Two cultures with an initial titre of  $10^{-7}$ , declined to  $10^{-1}$  after 18 and 21 days; thereafter, no growth was obtained. In the final experiment, the temperature varied between 19° and 26.5°C., and each culture was divided into two volumes: a series of 2 ml. quantities (which were each titrated in duplicate on only one occasion), and a 50 ml. quantity (which was serially sampled and titrated in duplicate each time). There was no difference between the two volumes in the decline of titre: initial titres of  $10^{-7}$  or  $10^{-8}$  were still as high as  $10^{-5}$  after 10 to 16 days and declined to  $10^{-2}$  after 31 days.

**Refrigerator Temperature.** Four parallel cultures to those in the first experiments above were kept between 4° and 11°C. There was virtually no decline from initial titres of  $10^{-7}$  or  $10^{-8}$  for about 14 days; thereafter, there was a slow fall to  $10^{-3}$  to  $10^{-1}$  after 50 days. In the parallel experiment to that at room temperature involving small (2 ml.) and large (50 ml.) volumes of culture, in which all samples were likewise titrated in duplicate, there was again no significant difference between the two volumes in the decline of titre, but now the titre persisted almost unchanged for 17 days and declined thereafter to  $10^{-2}$  or  $10^{-1}$  at 100 days.

*Survival on Solid Medium*

A liquid culture was seeded onto solid medium and the resulting colonies were passaged periodically to solid medium and liquid medium in parallel. Growth on solid medium and in liquid medium was obtained in the passages made 20 days after the primary seeding of the solid medium, but not after 25 days.

*Survival in Lung Tissue*

The main difficulty in these experiments was finding lung tissue that was apparently free from *Mycoplasma hyorhinis* and sufficiently free from bacteria, so that cultures made after the tissue had remained on the bench for a few hours were not rapidly overwhelmed with bacteria or, less rapidly but still effectively, overgrown with *M. hyorhinis*.

*Room Temperature.* With suitable lung material, an initial titre of  $10^{-6}$  remained high for 2 to 3 days and then fell sharply to  $10^{-2}$  after 7 days. The pieces of pneumonic tissue left on the bench in this way soon dried out and became dark brown.

*Refrigeration Temperature.* Parallel lung samples kept in the refrigerator yielded *M. suis* pneumoniae up to at least 11 days later, the initial titres remaining unchanged at  $10^{-6}$  for 9 days, and then falling to  $10^{-4}$  at 11 days. The pneumonic samples now showed no obvious signs of drying out until after about 7 days.

## DISCUSSION

These experiments show that *M. suis* pneumoniae can survive for many days at room temperature and for at least 7 to 14 weeks at between 4° and 11°C. in an artificial medium. In the natural medium of lung tissue, which was probably more comparable with the small quantities of nasal and tracheal exudate that might be transferred from farm to farm, *M. suis* pneumoniae survived for 1 week at room temperature and at least 11 days in the refrigerator, despite the drying-out process. It is possible that the mycoplasma might survive for longer periods in larger, moist pieces of pneumonic tissue or in other more favourable circumstances.

In assessing these experiments, it should be remembered that liquid medium is a less sensitive indicator for the presence of *M. suis* pneumoniae than the live, susceptible pig (Goodwin, 1972).

## ACKNOWLEDGMENTS

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## ERRATUM

*Research in Veterinary Science*, November 1971, Vol. 12. Page 596, second column, line 5:  
 For 'Coleotomy' please read 'Laparotomy'.