
NOTES

The Isolation of T-mycoplasma from Pneumonic Lungs of a Calf

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RÉSUMÉ

Les auteurs ont isolé une souche de Mycoplasme-T des poumons d'un veau Guernsey âgé de 2 mois et atteint de pneumonie. Aucun autre mycoplasme ou virus ne fut isolé de ces poumons. Une bronchiolite aiguë et un exsudat alvéolaire fibrino-cellulaire constituaient les principales lésions microscopiques. Une culture sur gélose-sang révéla la présence de *Corynebacterium pyogenes* qu'on considéra comme un microbe de sortie.

First isolations of T-strain mycoplasmas were made by Shepard (4, 5) from non-gonococcal urethritis in humans. Bovine isolates from the urogenital tract were reported by Taylor-Robinson *et al* (6) and from pneumonic calf lungs by Gourlay (1), who subsequently demonstrated the pathogenicity of his isolate by endobronchial inoculation of calves (3). This communication reports the first isolation of a T-mycoplasma from a calf in Canada.

The lungs from eight calves, one to eight months of age, that had pneumonic lesions at post mortem examination were stored at -70°C and were cultured for large colony mycoplasmas within one month. Seven to nine months after storage attempts to isolate T-mycoplasma and *Mycoplasma dispar* were made.

The liquid medium for T-strain isolation was essentially that described by Gourlay (1). PPLO broth base¹ with the addition of 0.002% phenol red was adjusted to pH 6.0 and for solid medium 0.65% agarose was added. The complete medium had final concentrations of 20% sterile, unheated horse serum², 2.5% aqueous yeast extract, 0.1% urea and 1000 units per ml penicillin G potassium. Thallium acetate was not used. Procedures used for isolation of T-mycoplasma from pneumonic lungs have been described (1).

Media and methods used for isolation of *M. dispar* were those described by Gourlay and Leach (2) but PPLO broth was substituted for Hartley digest broth³. Agar plates for T-mycoplasma and *M. dispar* were incubated at 37°C in an atmosphere of 10% CO_2 in hydrogen. Broth cultures were incubated aerobically at 37°C .

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Submitted December 14, 1971.

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²Grand Island Biological Company, Grand Island, New York, U.S.A.

³Oxoid brand, British Drug Houses, Toronto, Ontario.

Attempts to isolate other mycoplasma species were made using PPLO broth or agar supplemented with 1% yeast autolysate⁴, 0.1% glucose, 1000 units per ml penicillin, 1:4,000 thallium acetate, and 15% unheated horse serum. A tube of diphasic broth-agar medium and an agar plate were inoculated with triturated lung suspension. Three successive transfers to diphasic and solid media were made at 48 hour intervals before a specimen was considered negative. Incubation was at 37°C and agar plate cultures were placed in a moist atmosphere containing 5-10% CO₂.

A T-mycoplasma strain was isolated from the lung of a two month old calf. An alkaline colour reaction in the broth was first observed after 48 hours in the tubes containing 10⁻¹ and 10⁻² dilutions of lung suspension and after 72 hours in 10⁻³ and 10⁻⁴ dilutions. Subcultures, made as soon as a colour change was observed, produced an alkaline pH within 18 to 24 hours. Minute colonies appeared on solid medium after 24 to 48 hours and the pH became alkaline.

No other mycoplasmas and no viruses were isolated from this specimen, however, *Corynebacterium pyogenes* was recovered on blood agar plates incubated in 10% CO₂. The virus isolation attempts were made in cell cultures of embryonic bovine spleen.

No mycoplasmas were isolated from the other seven lungs, but it is not known what effect prolonged storage of tissue at -70°C has on the survival of *M. dispar* or other bovine mycoplasmas.

The T-mycoplasma strain was isolated from a dead female Guernsey calf from a group of 15 calves all of which had chronic pneumonia. Most calves responded to treatment with various antibiotics, including penicillin, streptomycin, tetracyclines and chloramphenicol. Six calves continued to cough, lost weight and died after a prolonged illness.

The lesions were confined to the respiratory system. The trachea and bronchi contained a copious amount of mucopurulent exudate. The apical, cardiac and lower two-thirds of the diaphragmatic lobes were firm, atelectatic and dark red.

Microscopically, the basic lesion in sections of antero-ventral areas was one of

bronchiolitis with alveolar collapse and marked fibrino-cellular exudation into the alveoli. Large numbers of multinucleated giant cells were evident in some alveoli.

The significance of the isolation of T-mycoplasma with regard to the etiology of the lesions described must be interpreted with caution. Endobronchial inoculation of T-mycoplasmas in three week old calves produced clinical signs of pneumonia in six of sixteen animals and pneumonic lesions were observed in fourteen (3). The characteristic microscopic lesions in these experimental calves were alveolar collapse, not usually accompanied by any alveolar reaction, and acute bronchiolitis. Proliferation of alveolar epithelial cells and the formation of multinucleated giant cells was not seen in the calves inoculated.

Attempts to isolate Parainfluenza-3 (PI-3) virus from the lung were unsuccessful and neither cytoplasmic nor intranuclear inclusions were observed.

C. pyogenes, also isolated from the lung of this calf, is usually associated with abscessation of the lungs and is generally considered to be a secondary invader.

ACKNOWLEDGMENTS

We wish to thank Dr. R. N. Gourlay for supplying a reference culture of T-mycoplasma and Dr. M. Savan for the virus isolation attempts.

REFERENCES

1. GOURLAY, R. N. The isolation of T-strains of mycoplasma from pneumonic calf lungs. Res. vet. Sci. 9: 376-378. 1968.
2. GOURLAY, R. N. and R. H. LEACH. A new mycoplasma species isolated from pneumonic lungs of calves (*Mycoplasma dispar* sp. Nov.) J. med. Microbiol. 3: 111-123. 1970.
3. GOURLAY, R. N. and L. H. THOMAS. The experimental production of pneumonia in calves by the endobronchial inoculation of T-mycoplasma. J. comp. Path. 80: 585-594. 1970.
4. SHEPARD, M. C. The recovery of pleuropneumonia-like organisms from negro men with and without nongonococcal urethritis. Am. J. Syph. Gonorr. Ven. Dis. 38: 113-124. 1954.
5. SHEPARD, M. C. T-form colonies of pleuropneumonia-like organisms. J. Bact. 71: 362-369. 1956.
6. TAYLOR-ROBINSON, D., M. H. WILLIAMS and D. A. HAIG. The isolation and comparative biological and physical characteristics of T-mycoplasmas from cattle. J. gen. Microbiol. 54: 33-46. 1968.

⁴Albimi Laboratories, Flushing, N.Y., U.S.A.