A MYCOPLASMA FROM ACUTE CONTAGIOUS CAPRINE PLEUROPNEUMONIA IN KENYA

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SUMMARY
A mycoplasma was isolated from acute caprine pleuropneumonia in Kenya. The organism could be differentiated serologically from the known strains of mycoplasma with which it was compared. When the organism was inoculated into goats it caused pleuropneumonia which was readily contagious, and from which the organism could be reisolated.

INTRODUCTION
There is confusion as to the nature of the causal mycoplasma of contagious caprine pleuropneumonia (CCPP); recent studies have segregated the mycoplasma into those that cross reacted by growth and metabolic inhibition tests with *M. mycoides* subspecies *mycoides* and those like the PGa strain of *M. mycoides* subspecies *capri* that did not (Al-Aubaidi, Dardiri, and Fabricant, 1972). In Kenya the causal mycoplasma had not been identified. An organism isolated from acute cases of caprine pleuropneumonia in Kenya was studied, therefore, and its pathogenicity for goats examined.

MATERIALS AND METHODS

Myoplasmas
Lung lesion material of 21 acute field cases of CCPP from 14 outbreaks yielded mycoplasma which were totally inhibited by growth-inhibiting serum to one isolate, F38. Two of these isolates, F38 and G69, were chosen for further study. F38 was purified a total of six times by serial subinoculation of a single colony, three times on media without bacterial inhibitors. G69 was purified similarly three times by serial subinoculation of a single colony, and shown in the growth inhibition test to be inhibited totally by F38 antiserum. High passage, freeze-dried cultures of F38 have been sent to the National Type Culture Collection, London, for further study.

Known mycoplasma from caprine pleuropneumonia used comparatively were the PGa strain (National Type Culture Collection 10137), Nigerian strains N108 and Vom (Cottew and Leach, 1969), strain Smith (Cottew, Watson, Erdag, and Arisoy, 1969), and F30 (MacOwan, 1976). The Gladysdale strain of *M. mycoides* subspecies *mycoides* was also included.

Experimental animals
All experimental animals were either reared in isolation on the laboratory farm at Kabete or brought from farms known to be free of contagious caprine and bovine pleuropneumonia. The goats were kept in isolation for at least 2 months prior to experiment. Experimental groups to be infected and control groups were balanced for breed, sex, and age.

Inocula
Infective inocula were 24 to 48 h log phase broth cultures of F38 and G69 which were free of contaminating bacteria and totally inhibited by F38 growth-inhibiting antiserum.
Experiments

1. Pathogenicity by the intratracheal-endobronchial route of inoculation. Eight goats of the Galla and cross-bred Galla type, ranging from 9 to 18 months in age, received 20 ml of F38 broth culture containing $10^{10}$ colony forming units (cfu) and 10 ml of sterile broth via the intratracheal-endobronchial route (Abdulla and Lindley, 1967). The organism was at the fifteenth pass in artificial medium. A further four goats were kept in close contact with the inoculated animals. A control group of eight goats was inoculated similarly with 30 ml of sterile broth. The experiment was terminated after 40 days.

2. Pathogenicity by contact. One goat received 0.5 ml of chloroform intravenously (Longley, 1951) followed 2 h later by 3 ml of broth culture containing $7 \times 10^7$ cfu of G69 at the tenth pass in artificial medium. Fifteen days post inoculation (pi) seven healthy goats were placed in close contact with the inoculated goat and three other goats were inoculated as above and added to the experiment.

As controls, one goat was inoculated with chloroform and sterile broth medium and seven healthy goats were maintained as contact controls.

Cultural methods

Media. For F38 viande-foie medium described by Al-Aubaidi and Fabricant (1968) was made from goat tissues and further modified by the addition of glucose, 0.5 per cent (w/v), Bacto-yeast extract (Difco), 0.1 per cent (w/v), thallium acetate, 0.5 per cent (w/v), penicillin, 100 iu/ml, glycerol, 0.5 per cent (v/v), and 50 per cent inactivated goat serum. The additives for solid medium were similar except for penicillin, 1,000 iu/ml, thallium acetate, 0.01 per cent (w/v), agar Noble, 1.7 per cent (w/v), and inactivated goat serum, 30 per cent (v/v).

For the other mycoplasma modified Newing’s tryptose media were used (MacOwan, 1975).

Cultural tests. Tests for fermentation of glucose, haemolysis of horse red blood cells, production of peroxide, reduction of methylene blue, liquefaction of inspissated medium containing goat serum, and growth in embryonated eggs were as described previously (MacOwan, 1976). Newing’s tryptose broth with one per cent glucose and 50 per cent inactivated goat serum was used for fermentation tests.

Serological tests

Complement fixation and agar gel diffusion tests. The methods of preparing antigens and rabbit antisera to all strains, and the agar gel double diffusion and complement fixation tests were similar to those described previously (MacOwan, 1976). For the preparation of F38 antigen it was necessary to grow the organism in medium containing goat serum.

Growth inhibition tests. The method employed was a modification of the procedure described by Clyde (1964). Serum wells 6 mm in diameter were used in place of filter paper discs soaked in serum. Growth inhibiting antisera to F30, N108, PG3, and M. mycoides subspecies mycoides (Gladysdale) were available (MacOwan, 1976). The antisera for complement fixation and agar gel diffusion tests prepared with strains Smith, Vom, and F38 possessed growth inhibiting activity.

RESULTS

Experimental results

1. Pathogenicity by intratracheal-endobronchial inoculation. Three of the eight