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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 PG_3 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

Mycoplasmas

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 PG_3 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 7×10^7 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, PG_3, 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

inoculated goats developed acute pleuropneumonia. Two cases became febrile for 6 days before death, 16 and 21 days pi respectively. Temperatures reached peaks of 41·6 °C and 41·7 °C but the goats were alert and active until 2 to 4 days before death when coughing, inappetance, and depression became apparent. The third case showed a similar phase, being febrile from day 8 to day 16 with a temperature peak of 41·5 °C on day 12. Coughing and partial anorexia developed during the febrile phase and remained until slaughter, 40 days pi, when the goat was emaciated.

Both acute cases showed fibrinous pleuropneumonia lesions similar to those observed in field cases of CCPP. Their chest cavities contained 100 to 150 ml of clear yellow pleural fluid. The chronic case had an extensive fibrous pleuropneumonia with fibrotic replacement of the left lung containing large foci of dry necrotic tissue in which the larger air passages alone remained patent.

Two of the in-contact goats became clinically affected, one dying 26 days post exposure, the other being killed on day 38. The former remained afebrile, developing a cough and inappetance 2 days before death, while the goat killed on day 38 showed symptoms similar to those of the inoculated acute cases. In the fatal case approximately 0·67 of the left diaphragmatic lobe was replaced with hepatised tissue showing a central necrotic area. The lesion was covered with yellow fibrin and adherent to the costal pleura. The lung of the second clinical contact case showed deeply penetrating hepatised lesions in the left apical and cardiac lobes.

At slaughter, 40 days post exposure, a third in-contact case had a total of 6 foci, 0·5 to 1 cm diameter, of variegated hepatised tissue distributed in the left lung and in the right cardiac lobe. This was a subclinical case. All affected goats showed grossly enlarged and oedematous mediastinal and bronchial lymph nodes.

Mycoplasma culturally and morphologically similar to F38 were reisolated from the lung lesions and mediastinal lymph nodes of the two inoculated acute cases and from the three in-contact cases. One isolate from each contact case was confirmed to be of the F38 type by growth inhibition test using antiserum to F38.

Inoculated and in-contact control goats were killed and found to have no lung lesions. No mycoplasma were isolated from the specimens of normal lung tissue examined.

2. Pathogenicity by contact. Fourteen days pi the first goat inoculated with culture showed a rise in temperature, coughing, and dyspnoea for 8 days prior to euthanasia on day 22. The temperature reached a peak of 41·4 °C. A further inoculated goat showed similar symptoms before slaughter 39 days pi. Of the remaining two donor goats one showed these symptoms and survived in emaciated condition, the other was unaffected.

Of seven in-contact goats five developed the same symptoms prior to slaughter 21 to 42 days post exposure, while two showed only partial anorexia prior to death 31 days post exposure.

The two inoculated goats slaughtered showed extensive lesions of acute fibrinous pleuropneumonia. The lung lesions were distinguished by the prominence of enlarged grey interlobular septae throughout the lung, not merely in the specifically affected consolidated tissue. Otherwise the lesions were similar to those observed in field cases of CCPP.

Of the contact cases six showed lesions of acute fibrinous pleuropneumonia with many of the characteristics seen in field cases of CCPP. The lungs of one afebrile contact case showed congestion and small areas of collapse not typical of CCPP.

Except for the atypical lung lesion in the one contact case mycoplasma were recovered from the lung lesion, mediastinal lymph node, and, where present, the

pleural fluid. All strains isolated were inhibited by hyperimmune serum to F38.

The control animals remained clinically unaffected. The goat inoculated intravenously with chloroform followed by sterile broth was killed immediately. The lungs were haemorrhagic with petechiae and small ecchymoses following the course of the interlobular septae. All other organs appeared normal.

Cultural results

F38 filaments were similar to those of *M. mycoides* subspecies *mycoides* (Gladysdale) except that they appeared to be more delicate. F38 reduced the pH of medium containing glucose by 0·5 to 1 unit, caused haemolysis, produced peroxide, reduced methylene blue, liquefied inspissated medium, and grew in embryonated eggs killing the embryos in less than 7 days.

Serological results

The agar gel double diffusion test showed each strain to have a minimum of two and a maximum of five antigens in common with any one of the others.

The complement fixation test enabled *M. mycoides* subspecies *mycoides* (Gladysdale) to be distinguished from the caprine strains. However, high titre cross reactions were found among the caprine strains.

The growth inhibition test separated the strains. PG_3, N108, and Smith were inhibited by their homologous antisera as well as by their heterologous antisera; F30, Vom, and *M. mycoides* subspecies *mycoides* (Gladysdale) were similarly interrelated; while F38 and G69 were inhibited only by F38 antiserum.

DISCUSSION

F38 and G69 caused fibrinous pleuropneumonia following inoculation of goats, and the condition was readily contagious to healthy goats. With the exception of the two inoculated cases where chloroform was injected and an afebrile contact case, the lung lesions of all experimental cases were macroscopically indistinguishable from those of field cases of CCPP.

Yedloutsching, Taylor, and Dardiri (1971) observed in-contact disease in a kid and complement fixation antibodies in kids suckling inoculated dams. Apart from this work, there are no reports of culture-induced pleuropneumonia causing clinical disease in contact goats. It is generally accepted that the causative mycoplasma of CCPP are difficult to isolate, and there is little information as to the frequency of isolation of any accepted strain. Mycoplasma of the F38 type were isolated from 21 acute cases from 14 outbreaks.

The findings of the complement fixation and agar gel diffusion tests indicate that F38 is related to *M. mycoides*. In addition the growth inhibition test indicated that F38 differed from PG_3, N108, Smith, F30, Vom, and *M. mycoides* subspecies *mycoides* (Gladysdale).

CONCLUSION

Following inoculation of goats a mycoplasma frequently isolated from acute field cases of contagious caprine pleuropneumonia induced a contagious fibrinous pleuropneumonia. While serological characters placed the organism in the species *M. mycoides*, the growth inhibition test indicated that it did not belong to Al-Aubaidi's groups 3 or 8.

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UN MYCOPLASME ISOLE DE PLEUROPNEUMONIE AIGUE DE LA CHEVRE AU KENYA

Résumé—Un mycoplasme a été isolé d'un cas de pleuropneumonie aiguë de la chèvre au Kenya. Ce germe a pu être différencié sérologiquement des souches connues auxquelles il a été comparé. Lorsqu'il est inoculé à des chèvres saines, il provoque une pleuropneumonie réellement contagieuse et est réisolé à partir des lésions.

UN MICOPLASMA AISLADO DE CASOS DE PLEURONEUMONIA AGUDA CONTAGIOSA CAPRINA EN KENIA

Resumen—Se aisló un micoplasma de casos agudos de pleuroneumonia caprina en Kenia. Los organismos se diferenciaron serologicamente de otras cepas conocidas de micoplasma con las cuales se compararon. Cuando los organismos se inocularon en cabras, la enfermedad se reprodujo y el organismo causal pudo aislarse nuevamente de los casos clínicos.