STUDIES ON CONTAGIOUS CAPRINE PLEUROPHLEUMONIA

Kenneth James MacOwan, B.V.M. & S., M.R.C.V.S.
This thesis has been composed by me, and describes my own work.
ACKNOWLEDGEMENTS

I wish to thank Dr G.R. Scott for his helpful advice and criticism, and Mr J.G. Tremlett for his active support and his continuing interest. I am also indebted to the Director of Veterinary Services, Kenya, for his permission to present my work in this form.

In addition I thank Miss J.E. Minette for technical assistance during the last part of this study. Funds made available by the Overseas Development Administration during this time greatly facilitated my work.
CONTENTS

SUMMARY

INTRODUCTION

LITERATURE REVIEW OF CONTAGIOUS CAPRINE PLEUROPNEUMONIA
The classic disease 3
Atypical syndromes of CCPP 13
The experimental disease 16
Pathogenic M. mycoides from CCPP 28
Experimental pathogenicity of caprine M. mycoides 42
Pathogenicity of M. mycoides subspecies mycoides of bovine origin for sheep and goats 52
M. mycoides from species other than cattle and goats 54
Serological relationships between caprine M. mycoides and between these organisms and M. mycoides subspecies mycoides 55
Other recent techniques used to investigate inter-relationships of the species M. mycoides 64

MATERIALS AND METHODS
Examination of field cases and experimental cases 67
Mycoplasma strains 71
Pathogenicity experiments 72
Serological methods 78
Media 83
Cultural methods 86
Morphological examination 90

RESULTS 92
Preliminary study of field cases 93
Cultural results 97
Pathogenicity of isolate F30 100
Pathogenicity of type cultures for goats 107
Pathogenicity of F38 and G69 isolates for goats 114
Serological results 125
Cultural comparison of F30, F38, and reference strains of the species M. mycoides 130

Morphology 135

DISCUSSION 143
Experimental pathogenicity of F30 and reference agents of the M. mycoides species 147
Experimental pathogenicity of F38 152
Serological results 157
Cultural results 162
Morphology 164

CONCLUSION 166

REFERENCES

APPENDIX
Acute cases of contagious caprine pleuropneumonia from outbreaks in Kenya were investigated. A mycoplasma, designated F38, was isolated in 41 cases from 30 outbreaks but no organisms identical to *Mycoplasma mycoides* subspecies *capri* represented by the reference strains PG3, Smith and N108, were recovered. Similarly no organisms identical to *M. mycoides* subspecies *mycoides* of caprine origin represented by strain Vom were isolated from acute cases.

F38 was pathogenic to goats by inoculation and the pleuropneumonia induced spread readily to goats in contact. Primary isolation of this organism proved difficult and growth in vitro was slow.

A second mycoplasma, designated F30, was isolated from three chronic cases of caprine pleuropneumonia. F30 was pathogenic to goats and sheep following inoculation but the disease was not contagious. This organism grew readily in vitro even at primary isolation.

Serological, biochemical, cultural, and morphological studies suggested that F38 and F30 were members of the species, *M. mycoides*. Complement fixation and agar gel diffusion test results emphasized the similarity of F30, F38, and reference strains of the *M. mycoides* species. Growth and metabolic inhibition tests indicated that F30 but not F38 belonged to Al-Aubaidi's Group 8. Moreover F38 differed from *M. mycoides* subspecies *capri* strains PG3, Smith, and N108.

A small preliminary trial yielded evidence that a degree of protection to the contagious disease was engendered by the prior inoculation of goats with F38 passaged serially in cultures.
Strains F30 and F38 were sent to three International Mycoplasma Reference Centres for further study. Reports placed F30 as a member of the *M. mycoides* subspecies *mycoides* in Al-Aubaidi's Group 3. Classification of strain F38 is in progress; if F38 proves to represent a new serotype, a name will be proposed.
The most economically important disease of goats in Kenya is known as contagious caprine pleuropneumonia (CCPP). The disease has been recognized in the country since the early years of the century (Mettam, 1929), although the aetiological agent has not been isolated.

There are an estimated 4 million goats in Kenya (Anon, 1972), and a survey currently in progress indicates there are probably many more (Fig. I). The species is mainly marketed for meat but dairy goats have been introduced. At the time of writing a 1 year old animal of 20 - 30 kilos live weight sells at £7 - £9 sterling. In recent years the goat population has increased and the disease is becoming more important. While the bulk of goats occupy the range areas of the country the rise in population may be attributed partly to the increase in the number of small holdings in new settlement areas, where the goat has a role in bush clearance. In the latter circumstances communal grazing and lack of fences predispose to the spread of a contagious disease.

In the range areas many of the herds belong to nomadic or seminomadic tribesmen, and control by movement restriction and quarantine would be difficult to implement. Most of the goats have to be transported over long distances to market at urban centres on the coast or inland. The inevitable mixing of herds and delays are also ideal for spread of a contagious condition. The most practical approach to control of CCPP in Kenya would be an effective culture vaccine; while an autogenous vaccine might be of assistance the difficulty in obtaining sufficient fresh affected tissue would be formidable.
In other countries there is general agreement that CCPP is caused by a mycoplasma of the species *Mycoplasma mycoides*. Mycoplasma belonging to *M. mycoides* subspecies *caninum* as well as to *M. mycoides* subspecies *mycoides* have been implicated in the aetiology of CCPP. The only reported contact transmission of the culture induced experimental disease related to a mycoplasma of the *M. mycoides* subspecies *mycoides* (Yedloutschnig, Taylor and Dardiri, 1971). However none of the cultured agents has been reported to cause readily contagious experimental disease.

*In vitro* cultivation of these agents is reported as either simple or very difficult. In view of the failure of earlier studies at Veterinary Research Laboratories, Kabete, the latter would seem more probable. However in the literature there is only tenuous evidence to indicate that any of the agents isolated caused the natural or field disease. The problem is confused further by the divergence of opinion throughout the literature on almost every aspect of the disease and its aetiological agents. Even the descriptions of the macroscopic appearance of typically-affected lungs vary.

It was therefore decided to study field cases of caprine pneumonia resembling classical descriptions of CCPP; when the syndrome was recognized, to narrow the study in an attempt to isolate mycoplasma, bacteria, and/or viruses; to characterize any such isolates; and to evaluate by experiment their significance in the aetiology of the condition.
KEY TO DISTRICTS

1. THIKA
2. KIRINYAGA
3. BUSIA
4. BUNGOMA
5. TRANS NZOIA
6. ELGEYO MARAKWET
7. KAKAMEGA
8. NANDI
9. KISUMU
10. KERICHO
11. UASIN GISHU
12. KISII
13. SOUTH NYANZA
14. SIAYA
15. NAKURU
16. BARINGO
17. WEST POKOT
18. TURKANA
19. MARCABIT
20. WAJIR
21. MANDERA
22. SAMBURU
23. ISIOLO
24. LAIKIPIA
25. MERU
26. GARISSA
27. NYANDARUA
28. NYERI
29. EMBU
30. KITUI
31. TANA
32. MURANGA
33. KIAMBU
34. NAROK
35. KAJIADO
36. MACHAKOS
37. NAIROBI
38. TAITA
39. KWALE
40. KILIFI
41. LAMU

Figure 1
LITERATURE REVIEW OF CONTAGIOUS CAPRINE PLEUROPEUMONIA

In Kenya and other countries where the disease occurs it is difficult, even with modern transport, to obtain specimens in suitable condition and from the right stage of the disease for optimal cultural study. A further problem arises from the absence of any practical curative or prophylactic measures which leads to severe losses. Even clinical cases, being killed for human consumption, for this reason it is often necessary to purchase even advanced clinical cases for laboratory investigation.
Descriptions of this disease have been reviewed in detail, from both a clinical and a pathological viewpoint, because they cover a wide span of time and place, with the inevitable difficulty that many excellent clinical descriptions are unsupported by adequate pathological data. This is compounded by the lack of suitable illustrations even at the macroscopic level. In addition, suitable cultural methods were often either not available or not feasible in the absence of the necessary laboratory facilities.

In Kenya and other countries where the disease occurs it is difficult, even with modern transport, to obtain specimens in suitable condition and from the right stage of the disease for optimal cultural study. A further problem arises from the absence of any practical curative or prophylactic measures which leads to goats in affected herds, sometimes even clinical cases, being killed for human consumption. For this reason it is often necessary to purchase even advanced clinical cases for laboratory investigation.
THE CLASSIC DISEASE

Occurrence

Contagious caprine pleuropneumonia (CCPP) was first described in 1873 by Thomas in Algeria (Turner, 1959). It was reported from South Africa in 1880, the French Pyrenees in 1888, India in 1889, Germany in 1894, Turkey in 1896, Kenya in 1915, Italy in 1916, Greece and Bulgaria in 1920, the Belgian Congo in 1921, Gambia and Nigeria in 1929, Syria in 1931, Colombo, Eritrea, and Uganda in 1933, Tanganyika, Sudan, French West Africa, and Morocco in 1934, and Spain in 1950. It has also possibly reached Brazil (Turner, 1959). It has been reported in Switzerland, Macedonia, Palestine, Egypt, Italian Somaliland, Tunisia, the Gold Coast, French Equatorial Africa, Mauritania, Ceylon, the Malay States, and U.S.S.R. (Longley, 1940, 1951; Shirlaw, 1949). To this list Cottew and Leach (1969) added South America, Iran, the Arabian Peninsula, Afghanistan, China, Burma, and Mongolia. However probably not all such reports can be accepted as relating to the true contagious caprine pleuropneumonia.

Prevalence

Mettam (1929) observed that in Kenya the virulence of the disease varied at different seasons, being worst during the wet months when animals were exposed to continual inclement weather by day and subsequently to the smoky, choking atmosphere of huts by night. It was very difficult to find indigenous goats suffering from the disease during the dry season, but chronic cases were observed in imported animals at this time, and in every instance there was evidence that infection was incurred during the wet season.
The disease in other countries was considered most prevalent in mountainous areas, for example, the Pyrenees, and the Kangra district of the Himalayas, but there was evidence that in Kenya it occurred from sea level to higher than 10,000 ft. In Greece and Italy also, the disease occurred at no marked altitude.

Longley (1940) and Shirlaw (1949) both mentioned a higher incidence of the disease in highland areas of India, and in addition Shirlaw (1949) recorded a higher incidence in relation to the cold season and monsoon season.

Christodoulou and Talartzis (1957) considered that the disease persisted in a latent form for a long time and produced overt symptoms in combination with factors such as a rigorous winter, poor nutrition, increased parasitic burden, and vaccination.

Bawa (1946) related the highest annual incidence to inclement weather, and Pillai (1965) also considered inclement weather could precipitate the disease.

Symptoms

Longley (1940), in India, gave the most comprehensive description of the symptoms shown by field cases of CCPP. Copious nasal discharge was commonly observed but it was almost always attributable to heavy infestation with larvae of *Oestrus ovis* which were present in over 60 per cent of the goats examined. However, from a study of 10 field cases in the Dharwar tract, Shirlaw (1949) found catarrhal inflammation of the upper respiratory tract with rhinitis and nasal discharge to be a specific feature of the disease and he failed to find larvae of *Oestrus ovis*. In many cases with even
advanced lesions no cough was evident. Generally the disease was afebrile although a transient rise of temperature to about 40°C was not rare, often occurring on the day of or a day or two preceding death. Solana and Rivera (1967) in Mexico also observed that pyrexia was not a consistent feature of the field disease. Usually appetite was unimpaired up to the agonal stage. Constant symptoms were loss of condition, weakness, and a staring coat, while in experimental pneumonias sudden dullness and listlessness were valuable signs. Dyspnoea was sometimes marked, particularly on exertion and in the latest stages, when the bleat also became weak and of peculiar timbre. With the onset of symptoms, if any, the course was rapid and death occurred usually within one week. Death was sudden, the animal being found dead in the morning or suddenly collapsing during the day and expiring in an hour or so.

In contrast to this description Bawa (1946), also in India, emphasized loss of appetite, cessation of rumination, and invariably a rise in temperature to approximately 41°C.

In Nigeria, Longley (1951) described peracute, acute, and chronic forms of the disease. In the peracute forms, fulminating consolidation of the lungs with pleuritic exudation completed the course of the disease in 3–5 days, sometimes as soon as 12–24 hours from the onset of symptoms. In the acute form the syndrome was an ordinary pneumonic one but the course was prolonged to 15 to 30 days. The chronic form developed from the acute, corresponding with resolution or encapsulation of the acute lesion. The disease in Nigeria was much more dramatic: sudden onset, dullness, pyrexia, anorexia, cough, costal tenderness, dyspnoeic respirations becoming stertorous
and oral, with consequent salivation leading to precipitate collapse and death from syncope. Sudden death with little or no premonitory signs was common to the disease in India and Nigeria.

Christodoulou and Talartzis (1957) observed lameness associated with arthritis and polyarthritis in addition to the symptoms above.

Mettam (1929) elaborated on the symptoms of the chronic form of the disease. Chronically-affected animals existed in an unthrifty or emaciated condition and generally suffered from an intermittent enteritis. Such goats coughed occasionally, especially on exertion. Some chronic cases suddenly relapsed, developed acute symptoms, and died. The cause of these breakdowns was not always evident, but they had been observed to follow contact with natural acute cases. Possibly some cases relapsed as a result of exposure to unsuitable environmental or atmospheric conditions.

Host Specificity
Several workers have found the disease to be specific for goats (Bawa, 1946; Turner, 1959), sheep apparently being unaffected.

Age and Sex Susceptibility
All ages and both sexes appeared equally susceptible to the disease (Mettam, 1929; Pillai, 1965).

Incubation Period and Course
There is little information on the incubation period of the disease in the field. Shirlaw (1949) mentioned the incubation period had been found to be 2–15 days; Christodoulou and Talartzis (1957) observed an incubation period of 5–6 days.

The course of the disease varied, but was never much longer
than 4 weeks. Christodoulu and Talartzis (1957) observed a course of 10 - 15 days in less acute fatal cases; Solana and Rivera (1967) found the course to vary from 2 - 5 days in fatal cases; Shirlaw (1949) reported that the duration of the disease was 2 - 12 days, although some animals might survive for longer periods, even 2 - 3 weeks; in an outbreak in the Dharwar River tract the course was reported as 3 - 8 days, while in another outbreak he mentioned 3 animals recovered, although recovery was exceptional. Bawa (1946) stated that in India the natural disease ran a course of 3 - 8 days although some animals survived as long as 2 weeks. Very few goats recovered.

Morbidity and Mortality

Reported mortality varies widely, that reported for Kenya (Mettam, 1929) being in the middle of the range (Table 1). Fewer authors have given morbidity figures, but those reported also vary widely (Table 1).

Immunity

It was reported that in India recovery from the natural disease conferred immunity (Bawa, 1946; Shirlaw, 1949).

Mettam (1929) observed that the immunity of native goats in the Belgian Congo contrasted with the susceptibility of native goats in Kenya and Uganda. He considered it possible that the disease had been introduced only recently east of the Great Lakes and the Mountains of the Moon.

Macroscopic Lesions

In India Longley (1940) described the condition in detail as found in Madras province. Of 54 cases studied, 43 manifested pleuritis,
Table 1. Morbidity and mortality of contagious caprine pleuropneumonia

<table>
<thead>
<tr>
<th>Morbidity (%)</th>
<th>Mortality (%)</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-20</td>
<td>10</td>
<td>Mexico</td>
<td>Solana &amp; Rivera (1967)</td>
</tr>
<tr>
<td>-</td>
<td>10*</td>
<td>Greece</td>
<td>Christodoulou &amp; Talartzis (1957)</td>
</tr>
<tr>
<td>-</td>
<td>50*</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>-</td>
<td>60-100*</td>
<td>&quot;</td>
<td>Stylianapoulo (1933) quoted by Longley (1951)</td>
</tr>
<tr>
<td>-</td>
<td>50-60</td>
<td>Algeria</td>
<td>Castelet (1906) quoted by Longley (1951)</td>
</tr>
<tr>
<td>-</td>
<td>50-60</td>
<td>Kenya</td>
<td>Mettam (1929)</td>
</tr>
<tr>
<td>-</td>
<td>50-60**</td>
<td>Africa</td>
<td>Curasson (1936) quoted by Shirlaw (1949)</td>
</tr>
<tr>
<td>64++</td>
<td>68++</td>
<td>India</td>
<td>Shirlaw (1949)</td>
</tr>
<tr>
<td>-</td>
<td>60-100***</td>
<td>&quot;</td>
<td>Bawa (1946)</td>
</tr>
<tr>
<td>30-40</td>
<td>60-100</td>
<td>Sudan</td>
<td>Pillai (1965)</td>
</tr>
<tr>
<td>-</td>
<td>90</td>
<td>Italy</td>
<td>Mori (1916) quoted by Shirlaw (1949)</td>
</tr>
<tr>
<td>-</td>
<td>up to 100</td>
<td>Nigeria</td>
<td>Henderson (1928)</td>
</tr>
<tr>
<td>-</td>
<td>up to 100</td>
<td>&quot;</td>
<td>Kearney &amp; Beaton (1927)</td>
</tr>
</tbody>
</table>

* 1 outbreak with 50% mortality and 2 outbreaks with 10% mortality
+ Seasonal variation from 60% in spring up to 100% in winter
** Generally, but could be up to 100% in some outbreaks
++ Data on 1 field outbreak
*** Reported findings of earlier workers
24 with 50 ml or more of effusion, and in 4 instances as much as 300 ml. He found that the frequency of left and right lung involvement was the same and that bilateral involvement was twice as common as unilateral, though the lesions were usually more extensive on one side. The diaphragmatic lobe was most frequently involved, followed by the cardiac lobe, the apical, and finally involvement of all 3 lobes.

The visceral pleura over a pneumonic lesion was consistently involved. Pleuritis was usually present on the costal pleura but was very variable in extent. In early cases a small focus of dry pleurisy would be evident, and well-marked congestion of the capillaries, which stood out in a clear network, was invariably present. All degrees from this to involvement of the entire pleural surface were observed. Where the lesions were extensive there was generally effusion, invariably serous or serofibrinuous. If liquid, coagulation of the effusion occurred within 5 minutes of opening the thorax.

Early lung lesions were either focal or diffuse, the focal lesion being single or multiple small zones of yellow to grey hepatization, slightly raised and clearly demarcated from the surrounding healthy lung. The diffuse lesion almost invariably involved the apical or cardiac lobes in part or entirely, being a frank oedema both of the alveoli and the interlobular septae. The lesion was resilient to the touch, usually in continuity with a hepatized area and, on incision, exuded clear, straw-coloured fluid which was highly virulent. This lesion was red, presenting a picture of alveolar oedema with marked dilatation and congestion of the capillaries of the alveolar walls and oedematous infiltration of the
Interlobular septae. Macroscopically the lesion was readily discernible but it was never as gross as in the bovine disease. With this type of lesion spread was via the interlobular septae, while the focal type of lesion appeared to spread by progressive, non-selective involvement of all surrounding lung tissue. At autopsy there was generally partial or complete hepatization of the lobes with varying extension by one or other of the above early lesions. The hepatization was either red or grey, but the traditional red hepatization with rupture of capillaries and accumulation of erythrocytes in alveoli did not occur. In extensive and late lesions the main portions were extremely friable and dry, and the central portions were frankly necrotic. In a few instances inextensive but unmistakeable specific lesions were found undergoing fibrosis and encapsulation. A marbled appearance of the lung resembling the typical bovine lesion was observed on very rare occasions only and was in no way characteristic of the caprine disease.

Other lesions included swollen, oedematous, and sometimes congested mediastinal lymph glands, and in one case splenomegaly. In one case serofibrinous pericarditis was observed, the pericardium being thickened, oedematous, and containing a considerable quantity of coagulated effusion. In addition there was marked oedema over the coronary grooves. In 2 cases very oedematous thickening of the pericardium was observed, but with no evident involvement of the serosal surfaces or abnormal effusion. In general no lesions other than those in the thorax were present.

Shirlaw (1949) compiled a similar description of the macroscopic lesions as a review of the findings of previous authors working in
other parts of the world. He mentioned volumes of pleural fluid from 0.5 to 2 litres; that hepatized lesions projected above the lung surface; and that the bronchi contained clear exudate which clotted immediately at death. His account differed from Longley's in the following statement: 'The appearance of the lung is similar to that seen in lung affected with contagious bovine pleuropneumonia; it is marbled and forms a mosaic pattern as a result of variegation of the lobules which are deep red, bluish, wine coloured, or greyish and sharply demarcated by the interlobular connective tissue which is prominent and distended by exudate, sometimes this infiltration is absent.' In addition he recorded that the pericardial sac usually contained exudate, sometimes in considerable quantity, while in some cases the mediastinal lymph nodes were not only swollen but infiltrated and haemorrhagic. From a study of 10 field cases he observed pericarditis was a frequent finding. No lesions were found in the abdominal viscera except for those of parasitic gastroenteritis, to which he attributed the severe diarrhoea seen in the course of the disease.

In Kenya, Mettam (1929) was the first to describe the disease unequivocally. He observed that hepatized lesions exuded a bloody, watery fluid when broken, and he emphasized the frequency of pleurisy. The fibrin deposit was soft, slimy, up to 3 cm thick, and full of lacunae containing the characteristic exudate. In peracute cases it was easily removed from the lung, but it was more firmly attached in older cases. In bad cases the fibrin covered the entire lung extending onto the pericardium. Where pneumonia was bilateral the deposit and exudate were generally, but not constantly, bilateral
also. The citron-yellow, odourless, pleural exudate measured as much as 0.5 litre, being unilateral or present in both mediastinal cavities. In cases with a course of 10 days or more, there were adhesions between the parietal and visceral pleurae. These were soft and gelatinous in nature, becoming more dense and fibrous in older cases. The parietal pleura could be quite normal or very injected, somewhat thickened, moist, and covered with adhesions whose nature depended on the type and course of the disease.

He observed a flabby, soft appearance of the heart muscle enclosing much-dilated cavities, generally in association with chronic cases. His description was similar to that of Longley (1940). Mettam stated 'When resolution is not complete fibrosis follows hepatisation and the anatomical picture may superficially resemble the lung in bovine pleuropneumonia although there was no difficulty pathologically in distinguishing between the bovine and caprine diseases.'

He elaborated on the chronic form of the disease. There might be no pleurisy but in the majority of cases the lungs were firmly attached to the chest wall by dense, tough, fibrinous adhesions, which generally arose from the visceral pleura covering a normal lung. In chronic cases no exudate was ever noticed. In a few cases a bilateral fibrous pleurisy existed independently of any concommitant pneumonia although generally both were present. He observed that, if the animal survived, the fate of an acute pneumonia was a chronic fibrous pneumonia without suppuration, necrosis, or gangrene. In a few cases almost complete resolution occurred so that respiratory function of diseased lung tissue was restored.

Neither Mettam (1929) nor Stylianopoulu (Shirlaw, 1949) agreed
with the distribution of lung lesions recorded by Longley (1940) in India. Stylianopoulou (Shirlaw, 1949) found that as a rule the right lung was chiefly affected and much less commonly both lungs; while Mettam (1929) found the right lung affected in 40.5 per cent of cases, the left lung in 23 per cent, and the remaining 36.5 per cent with bilateral pneumonia, lesions being most commonly found in the base of the main and cardiac lobes.

Bawa (1946), from his study of 49 field cases, gave an account of the macroscopic lesions which was very similar to that of Longley (1940). In old and extensive cases he found portions of the affected lung became friable, dry, and necrotic. In addition this author did not observe enlargement of the spleen.

In these detailed accounts of the macroscopic lesions of CCPP the most significant difference was the occurrence of lobulation. Longley (1940) and Mettam (1929) considered lobulation was a minor facet, while Shirlaw (1949), and Kearney and Beaton (1927) record lobulation as a prominent feature, so that the overall pneumonic lesions were similar to those observed in contagious bovine pleuropneumonia (CBPP); Cottew and Leach (1969) maintained that there was massive oedema and the interlobular septae were frequently dilated with serous fluid.

Christodoulu and Talartzis (1957) observed arthritis and lameness; in some cases polyarthritis severely affecting posture; affected joints contained serofibrinous liquid.
ATYPICAL SYNDROMES OF CCPP

Agents classified as *E. mycoides* subspecies *capri* have been isolated from several different disease syndromes and lesions:

**Oedema or Sparta Disease**

In Sparta, Melanidi (1951) continued the study of 'oedema disease of goats' initially described by Debonera (1937), who attributed the syndrome to *M. agalactiae*, while Edward (1953) found the organism to be serologically as well as culturally and biochemically related to the organism of goat pleuropneumonia. The characteristic symptom was subcutaneous infiltration and oedema accompanied by heat and pain, affecting the head, especially the lower jaw, forelegs, sternum, thigh, and sometimes the area around one teat. The animals became febrile, with a temperature of 41 - 42°C, showing general weakness, rapid emaciation, and nearly total inappetance, together with lameness. The course of the disease was 3 - 5 days and it was always fatal. Post-mortem examination revealed subcutaneous oedema; exceptionally the heart was slightly hypertrophied, although ecchymoses and pericardial exudate were not uncommon; but the liver was generally unaffected, except when the oedema primarily involved the abdominal wall the liver surface revealed haemorrhagic spots. Several lymph nodes, especially those draining oedematous areas, were enlarged and reddened; the spleen was slightly enlarged; and the kidneys usually showed lesions of acute nephritis. The intensity of renal haemorrhage varied; scattered haemorrhages were generally present in the pelvic region. The nephritis caused urinary retention and this was reflected by changes in the blood.
 Conjunctivitis

In Connecticut, Jonas and Barber (1969) isolated a strain which they referred to as *M. mycoides* subspecies *capri* from the right eye of a goat recovering from experimental brain surgery at the Yale University School of Medicine. The animal was depressed and had partial inappetance. The right periorbital tissues were oedematous and a mild conjunctivitis was noted. The goat also had a temperature of 41°C, a supple neck with no signs of meningitis, proper locomotion with no signs of ataxis or lameness, and normal heart and lung sounds on auscultation. Following 5 days of treatment with penicillin and dihydrostreptomycin the goat made an uneventful recovery with no obvious residual effect.

North American Syndromes

Yedloutschnig, Taylor, and Dardiri (1971) described 4 highly-pathogenic mycoplasma, isolated from several conditions in different areas of the United States. These isolates were biochemically and serologically indistinguishable from caprine strains Connecticut, Mexico and Vom. The first isolate came from 2 kids recently introduced to a small herd in Pennsylvania. The kids developed lameness, a stiff gait, and a temperature of approximately 39.3°C. Necropsy revealed consolidation of the apical, cardiac, and diaphragmatic lobes of the lung, and mycoplasma were isolated from lung and heart sections as well as from swabs of pericardial and synovial fluid. The second isolate was recovered from the eye swabs of 5 - 10 goats with keratitis. The animals were kept for research by the National Institute of Health, Otisville, New York. A third mycoplasma was isolated from a hard, grey and red mottled mass, 5 - 6 inches long, in the cervical region of a goat in Maryland. The animal had been recently purchased and added to a milking herd of 35. The fourth
A mycoplasma investigated came from the synovial fluid of the swollen hock joint of a 14-month-old goat in a different part of Maryland. This goat belonged to a herd of 12.

Pearson, Rokey, Harrington, Proctor, and Cassidy (1972) isolated a mycoplasma with pathogenicity similar to \textit{M. mycoides} subspecies \textit{capri} from young kids. This agent was classified as \textit{M. mycoides} subspecies \textit{capri} by the Plum Island Animal Diseases Laboratories. Eleven kids showed inappetance, swollen joints, recumbency, diarrhoea, and body temperatures of 41 - 42°C prior to death within 5 days. Eight adults in the herd were unaffected.

Post-mortem examination revealed subcutaneous oedema, consolidation of pendant parts of the lungs, and fibrinous pericarditis. Regional lymph nodes were moderately enlarged and haemorrhagic. There were petechial haemorrhages in the calices of the kidneys. Arthritic lesions, which were most severe in the hip and hock joints, consisted of erosion of articular cartilages and accompanying periarthritis with deposition of oedema and fibrin around the joints.

\textbf{Mastitis and Arthritis}

In France, Perreau, Cuong, and Vallee (1972) investigated an outbreak of mastitis and arthritis in a herd of 100 goats of which 30 were affected. From the milk of 3 goats they isolated a mycoplasma of the \textit{M. mycoides} subspecies \textit{capri} group.
Inocula

The symptoms and lesions following inoculation of crude exudates and mascerated lesions from field cases of CCPP were most comprehensively described by Longley (1940, 1951) and Shirlaw (1949).

Longley (1940) employed diseased lung tissue and pleural effusion from 5 field cases of the classical disease. The inoculum was prepared by aseptically collecting lung effusion and adding suitable portions of minced, diseased lung tissue. This pneumonic lung emulsion (PLE) was mixed with an equal volume of cold tyrodes solution, pH 7.4, and agitated for a few minutes, strained through fine mesh wire gauze, bottled, and stored at 0 - 5°C. On all occasions the material used as inoculum was collected from animals immediately after death.

Shirlaw (1949) used as inocula mixed saline extracts of pneumonic lung and/or pleural exudate from 2 field cases.

The Disease Initiated by Different Routes of Inoculation

Subcutaneous and intramuscular inoculation. Longley (1940) found that inoculation of 5 ml PLE intramuscularly in the thigh or subcutaneously in the shoulder caused lameness within 2 days followed during the next 2 - 3 days by progressive swelling at the site of inoculation. The goats became disinclined to stand or feed, no fever developed, and death followed severe prostration with a subnormal temperature. In India he advocated ear-tip inoculation of virulent material as a prophylactic procedure. Since this route of inoculation was relatively innocuous, it is noteworthy that subcutaneous inoculation over the ribs caused a local reaction which could give rise to lung lesions by contiguous spread. His
observation was substantiated by Bawa (1946), also in India.

Shirlaw (1949) found subcutaneous inoculation in the neck and ear tip in some cases caused specific pulmonary lesions in addition to progressive oedema at the site of inoculation and temperatures rising to 41 - 42°C. He found the temperature rose within 24 hours of inoculation and remained high throughout the 3 - 12 day course of the disease, falling to subnormal just before death. The disease following subcutaneous ear-tip inoculation of lymph 'virus' was often rapid in course and somewhat septicaemic in character. In a few cases pneumonia was absent and the lesions suggestive of a pure septicaemia.

Longley (1951) observed the Nigerian agent to be much more virulent than the one he had studied in India. After passage, the Nigerian agent was consistently lethal by 3 - 4 days post inoculation. In addition, subcutaneous inoculation of the Nigerian agent could give rise to pulmonary lesions. This confirmed the work of previous authors in Nigeria: Kearney and Beaton (1927) described a marked thermal reaction and death within 5 - 6 days following subcutaneous or intramuscular inoculation of spleen pulp, heart blood, lung emulsion, or fluid from natural cases of CCPP. Beaton (1931) observed specific pulmonary lesions following subcutaneous inoculation, and he mentioned that pleurisy could arise as an extension of the reaction following inoculation over the ribs. Beaton (1931) emphasized the frequency of splenic enlargement in goats inoculated by this route.

From Longley's works (1940), on post-mortem examination these lesions presented massive oedema of the entire region with enormous infiltration of clear, straw-coloured effusion into the intramuscular and subcutaneous tissues; extensive necrosis of the muscles, which
were either pink or yellowish-grey; marked congestion of the small vessels of the subcutis and intermuscular septae, the dilated vessels standing out in a clear network in the oedematous bed; and oedematous enlargement of all regional lymph nodes. Though fluid on incision, coagulation of the effusion occurred within 2 or 3 minutes of its liberation. The lesions extended from the croup to the hock in the hind limb, where they were rather more advanced than in the fore limb. There was no gas formation and no smell.

The lesions were passaged in goats by inoculation of fresh oedema fluid. This myositis was later studied further by Hakioglu and Bogrun (1958) and Nagi, Sharma, and Bhalla (1967).

Mettam (1929) found that the subcutaneous inoculation of the juice expressed from pneumonic tissue caused only a febrile reaction. Abdulla and Lindley (1967) and Lindley and Abdulla (1969) in the Sudan found that virulent lung material and/or exudate given subcutaneously to 5 goats caused only a transient temperature reaction in 2 cases. Pillai (1965), also in the Sudan, obtained interesting results following inoculation of PLE; although the goats showed a rise in temperature and a local reaction similar to the above, when the inoculum was mixed with penicillin there was no reaction.

Similarly he observed no swelling after intramuscular inoculation of pleural fluid or blood from febrile cases.

Inoculation via the respiratory tract or lungs. Pulmonary lesions have been induced experimentally by several methods. The most natural procedures were exposure to aerosol inoculum, intranasal insufflation, and intratracheal endobronchial inoculation, the last procedure being the most quantitative. Longley (1951) readily transmitted the disease by disseminating PLE into small huts housing
experimental goats. He found age did not influence the susceptibility of goats using this route of inoculation. Bawa (1946), Karib (1958) Longley (1940, 1951), Pillai (1965), and Shirlaw (1949) employed intranasal insufflation successfully. Mettam (1929) mentioned a method of intrabronchial insufflation but gave no details of the technique. Abdulla and Lindley (1967) described the intratracheal endobronchial method and found this procedure to be more satisfactory than a form of aerosol inoculation. Other routes successfully employed included intratracheal (Longley, 1951), intrathoracic and intrapulmonary inoculation (Beaton, 1931; Heikkila and Ozkal, 1953; Mettam, 1929), and techniques involving coerced localization of the inoculum in the lungs following intravenous injection (Longley, 1940, 1951). These methods were designed to cause artificial pulmonary infarction by intravenous injection of sterile lung fragments or chloroform prior to injection of the inoculum. Shirlaw (1949) failed to cause the disease by a similar method whereby fragments of diseased lung were inoculated intravenously. Abdulla and Lindley (1967) failed to cause disease in 2 goats inoculated by the intrathoracic route with pleural exudate from field cases; they found the intratracheal endobronchial route to be the only successful method.

Apart from Beaton (1931), the authors found they reproduced the natural disease experimentally. The former stated 'A possible further theory is that contagious pleuropneumonia resembles swine fever and probably many other ultravisible diseases, in that a filterable virus and a bacille de sortie work in association with each other to produce the natural disease, and that the bacille de sortie in the case of contagious pleuropneumonia of goats is the organism under discussion.' It was noteworthy that Beaton's original inoculum came
from the pleural exudate of a typical case of CCPP. Longley (1951) considered that Beaton's work related to the specific disease.

Contact exposure. In view of the overriding importance of contact transmission to the understanding of a contagious disease all experiments to study contagious spread of CCPP are described together.

Longley (1940) observed contact transmission of CCPP to 1 of 7 healthy goats exposed to natural cases of CCPP. He also exposed 5 healthy goats to 30 experimental cases of CCPP, 24 of which showed lesions of the disease at post-mortem examination. Disease was induced in the donor animals by insufflation with pneumonic lung emulsion from field cases. After 6 weeks the 5 healthy goats were subcutaneously inoculated with the same inoculum and reacted fatally. In Nigeria, Longley (1951) exposed 26 healthy goats to contact with 38 experimentally infected donor animals. Such contact was maintained for the majority of the recipient group for 28 to 31 days, and for most of this period there were more donor animals than healthy contact goats under experiment. He observed contagious spread of CCPP to 4 contact animals: 2 died of the infection 11 and 23 days after exposure; 1 showed symptoms and involuting lesions at slaughter, 28 days after exposure; and the fourth had involuting lesions at slaughter 21 days post exposure. He failed to find airborne infection or residual infection in a box vacated by the donor animals in the contact experiment. He concluded that the term 'contagious' was proper to the specific disease.

Shirlaw (1949) observed contact transmission, airborne transmission, and transmission by indirect contact using donor goats experimentally affected following inoculation of lymph virus. Two
out of 4 goats kept in contact with 4 donor goats developed the disease, 1 dying 94 days after exposure, while the other was killed 101 days after exposure. The causal organism was isolated from both cases. In a second experiment, a healthy goat was tied alongside an infected animal and died 25 days after exposure. The third experiment comprised 2 healthy goats tied 10 ft and 20 ft from an infected goat and provided with separate food and water. Both healthy goats developed pleuropneumonia and died 11 days after exposure. In addition infection was shown to linger in sheds for at least 1 month after the last fatal case. The apparent ease with which this author performed these experiments contrasted with the work of Longley (1951).

Beaton (1931) exposed 2 healthy goats to challenge by 2 experimentally affected goats inoculated with culture at the 17 and 18 in vitro passage respectively. Both healthy goats were unaffected, and following inoculation with virulent culture died showing typical symptoms.

Other routes of inoculation. Beaton (1931) recorded fatal febrile disease following intraperitoneal inoculation of goats, with death at 6 days post inoculation. The subcutaneous tissues presented a ring of slight congestion with some fibrous thickening at the point of entry of the needle into the peritoneal cavity. The omentum, serous coat of the intestine, capsule of the liver, kidneys, and spleen were all covered with a fine film of slightly tenacious pus. Several hundred ml of thin white fluid were found free. The spleen was swollen and usually adherent to the diaphragm and rumen by white coagulated lymph. Small pneumonic lesions were sometimes present.

Mettam (1929) found that intravenous inoculation of pneumonic material caused a temporary febrile reaction without the production
of clinical pleuropneumonia. Pillai (1965) also failed to reproduce disease by intravenous inoculation of whole blood from a febrile case.

In contrast, Longley (1951) recorded acute, fatal disease following intravenous inoculation of virulent effusion from a local reaction into 5 goats. Three days post inoculation all 5 were very ill, but with no well-defined syndrome. Two were destroyed on the fourth day. The first goat had swollen oedematous lymph nodes, early specific lesions in the lung, pericarditis and myocarditis, and the heart showed the parboiled appearance of the coagulative necrosis seen in somatic muscles involved in local reactions. In the second animal there was specific localization in the popliteal region of the left leg. In the course of the next 8 days, 2 of the remaining goats died, and 1 was destroyed. Autopsy revealed in the first goat pericarditis, localization of disease in the left stifle region, hyperaemic circular patches of oedematous thickening in the wall of the rumen and in the second goat arthritis of the right shoulder joint, with coagulated effusion in the joint cavity. The third goat showed pericardial hyperaemia, splenomegaly, peritonitis with clear effusion, multiple foci of necrosis in liver, and a thickened and oedematous circular focus 5 cm in diameter involving musculature of reticulum and four similar foci in the small intestine, 2.5 - 7.5 cm long, all within about 0.5 m of bowel. The enteric lesions were all alike, red, oedematous, and with a diphtheritic tendency to separation of the mucous membrane. Each involved a complete cylinder of bowel.

The occurrence of the enteric lesions following intravenous inoculation was confirmed in 2 further experiments. The author concluded that following intravenous inoculation there was no marked predilection for the lungs.
In Mexico, Solana and Rivera (1967) also reproduced the disease in goats by intravenous inoculation of pleural exudate. The incubation period varied from 3 – 8 days. The first symptoms were depression, holding the head down, and failure to react to external stimuli. The temperature during the first days remained normal; on occasion it rose to 41°C a day before death. Fast breathing was a common finding, and after onset of symptoms abdominal respiration and serous nasal discharge usually developed. Terminally foamy exudate dripped from the half-open mouth. The neck at the site of inoculation was swollen to 2 to 3 times the normal size. In some inoculated goats there was an increase in red blood cells. In some animals there was leucocytosis and in others leucopenia. Differential counts showed a predominance of neutrophils.

At autopsy all goats showed congestion, oedema, and cellulitis at the site of inoculation; and grey and red hepatization of the lungs, usually localized to one lobule. The bronchi, bronchioles, and trachea were filled with foamy exudate. Up to 350 ml of clear yellow pleural exudate was present, and the pericardial sac was filled with liquid. Where the course was prolonged, a thick layer of fibrin covered the lungs and pericardial sac. The bile vesicle was swollen 2 to 5 times its normal size. The tissues about nerves, and apparently the nerves themselves, of the brachial and femoral plexuses showed petechiae and ecchymoses. However since the somatic tissues about the neck were inoculated it is debatable whether these experiments can be considered as examples of intravenous inoculation. No other author appears to have recorded nerve involvement.

Longley (1951) described extensive cutaneous elephantiasis
following intradermal inoculation. The outstanding feature of the lesions was their strict confinement to the skin. Affected skin was up to 5 cm thick with straw-coloured effusion and coarse hyperaemic blood vessels. All regional lymph nodes were greatly swollen, oedematous extension conspicuously following the lymph drainage.

**Incubation Period and Course**

The incubation period for the experimental disease showed a wide variation. In Nigeria, Longley (1951) found that the incubation period varied according to the route of inoculation. Mean survival periods following intratracheal inoculation, intranasal inoculation, and inhalation were, respectively, 7, 12.1, and 12.6 days. The course of the disease was rarely more than 2 days. Using various routes of inoculation he found the incubation period ranged from 3-24 days. Longley (1940) recorded an experimental incubation period of 8 - 28 days, normally 12 - 15 days, Mettam (1929) 4 - 16 days, and Shirlaw (1949) 3 - 21 days.

The course of the experimental disease also showed variation. Mettam (1929) observed the course to be as short as 3 days, and Shirlaw (1949) 3 - 14 days. In many cases, for example, Longley (1951), authors stated the survival period post-inoculation rather than the incubation period and course.

**Infected Tissues**

Mettam (1929) considered the 'virus' was present only in the lungs and thorax at the very beginning of the disease. He found it impossible to transmit the disease with material from chronic cases and suggested the 'virus' was no longer present. Longley (1951)
also considered chronic lesions to be non-infective, and in addition he found recent, fully-hepatized lesions to be deficient in this respect. He always attempted cultivation of the aetiological agent from the oedematous edge of the lesions. In 1940 this author prepared his inocula from the affected goats only on the day of death. This procedure was simulated by Bawa. Both these authors found that heating their inocula for 30 - 45 minutes at 55 - 60°C rendered them non-infective.

Abdulla and Lindley (1967), Bawa (1946), Beaton (1931), Heikkila and Ozkal (1953), Kearney and Beaton (1927), Lindley and Abdulla (1969), Longley (1940, 1951), Mettam (1929), Pillai (1965) and Shirlaw (1949) transmitted disease with pneumonic lung and/or pleural exudate from field and/or experimental cases, and with other infective tissues derived from experimentally-infected animals. Beaton (1931), Kearney and Beaton (1927), Longley (1940, 1951), and Shirlaw (1949) found experimental subcutaneous lesions and/or oedema fluid to be infective. Beaton (1931), Kearney and Beaton (1927), and Shirlaw (1949), in contrast to Pillai (1965), found blood taken at the height of fever to be virulent. In addition the first 2 references describe the infectivity of spleen from affected goats, while Shirlaw (1949) found pericardial fluid from an experimentally-infected sheep to be virulent for goats. Shirlaw (1949) considered it was possible that other tissues harboured the 'virus', by way of septicaemic localization, especially following subcutaneous inoculation.

Heikkila and Ozkal (1953) found pericardial fluid to be virulent but inoculation with heart blood and spleen gave variable results. Mettam (1929), Longley (1940), and Shirlaw (1949), were able to transmit disease with Seitz filtrates of infective material in contrast
Species Susceptibility

Sheep. Beaton (1931), Kearney and Beaton (1927), Longley (1940, 1951), and Shirlaw (1949) found their inocula virulent to sheep following subcutaneous or intramuscular inoculation, causing, in the main, symptoms similar to those following inoculation of goats by these routes.

Longley (1940) and Shirlaw (1949) described experiments in which sheep were insufflated with virulent material. Longley (1940) failed to cause disease in 5 sheep by this route in contrast to Shirlaw (1949), who recorded 1 case of pleuropneumonia following insufflation of 5 sheep. This animal died 16 days post infection, after a course of 4 days. Autopsy revealed acute pleuropneumonia, with exudative peritonitis and haemorrhagic gastroenteritis. One of the other 4 sheep developed a transient thermal reaction.

Abdulla and Lindley (1967) and Lindley and Abdulla (1969) failed to cause disease in sheep following inoculation by the intratracheal endobronchial route. Mettam (1929) and Bawa (1946) stated sheep were immune to experimental infection, without detailing the experimental techniques employed.

Intravenous inoculation of 3 pregnant sheep caused acute disease by the fourth day, with depression, anorexia, and refusal to lie down (Longley, 1951). Death occurred at 7 and 8 days post inoculation. At autopsy, in all animals the back from the withers to the croup was involved in a specific type reaction, the musculature being rigid with a coagulative necrosis, the entire area coarsely hyperaemic and massively oedematous. In 2 cases, clots of fibrin were present in the amniotic fluid, the allantois exhibited focal thickening.
radiating from the cotyledons, and the foetuses had numerous subcutaneous haemorrhages, from petechiae to extravasations the size of a sixpence, together with subcutaneous oedema and anasarca, especially in the cranial region. The thorax and pericardium were filled with serous fluid. The presence of the infective agent in 1 foetus was demonstrated by subcutaneous inoculation of goats.

Laboratory animals. Apart from Shirlaw (1949) no author has reported any reaction following inoculation of laboratory animals. Shirlaw (1949) observed fever in 1 guinea pig, following subcutaneous inoculation, starting 8 days post inoculation and continuing for 1 week before returning to normal.

Other species. Virulent tissues from cases of CCPP have been shown not to cause disease in cattle, buffalo, horse, ass, pig, guinea pig, white rabbits, and mice (Abdulla and Lindley, 1967; Beaton, 1931; Kearney and Beaton, 1929; Lindley and Abdulla, 1969; Longley, 1940, 1951; Mettam, 1929; Shirlaw, 1949).

Morbidity and Mortality

The morbidity of the experimental disease was between 75 and 92 per cent (Beaton, 1931; Longley, 1940, 1951; Mettam, 1929). Longley (1940) found 75 per cent of goats inoculated by insufflation were affected. Longley (1951) found the mortality to be 100 per cent during the first 5 passes of the Nigerian agent.
Just as some earlier descriptions of this disease lack appropriate bacteriological control, others lack sufficient clinical and pathological data. The latter is nowhere better illustrated than by PG₃, the world reference strain. The only association this strain bears to the natural disease being isolation from 1 case in Turkey (Cottew and Leach, 1969). Other reference strains considered the same as PG₃ bear an equally tenuous relationship to the natural disease.

Several authors have isolated pathogenic mycoplasma directly in lesions and exudates of field cases of CCPP (Abdulla and Lindley, 1967; Christodoulu and Talartzis, 1957; El Nasri, 1967; Pillai, 1965; Provost, 1966; Solana and Rivera, 1967), while others isolated the organism from the experimental disease induced with pathological material from field cases (Beaton, 1931; Longley, 1951; Shirlaw, 1949) (Table 2).

Organisms Isolated from the Experimental Disease

The most comprehensive series of animal experiments, particularly with goats, was carried out by Beaton (1931), Longley (1951), and Shirlaw (1949). However even these authors conducted only a limited number of experiments before finding the disease induced by their organism to be the same as that induced by exudates and lesions. They claimed isolation of their agents from the experimental disease a number of goat 'passes' from the original field case. This is a very important point because they may have isolated potential pathogens from apparently healthy experimental animals.

Beaton (1931) found the same disease following inoculation of culture or virulent tissues by the subcutaneous, intraperitoneal, and intrapulmonary routes. He described the typical disease
following each route of inoculation with limited numbers of animals and without details of those experiments initiated by culture. Shirlaw (1949) stated 'isolation was attempted in Bennett's broth with 10 per cent added goat serum. The first attempts sowing the tubes of this medium with a few drops of Berkefeld V filtrate of local lesion or pleural exudate gave indefinite results, but later, as the virulence of the lymph virus enhanced and became "fixed", it proved simple and certain to obtain primary cultures with such filtrates.'

Shirlaw (1949) did not detail experiments with his cultures, merely stating 'The proof that the organism we have isolated is the causal agent of contagious pleuropneumonia of the goat in India is that virulent and recently isolated cultures whether insufflated or subcutaneously inoculated consistently reproduced the disease in healthy goats and that the organism can uniformly be reisolated from the lesions produced.'

Longley (1951) also isolated mycoplasma from artificially-induced disease at 6 different goat 'passes' and from 10 different individuals. He reproduced disease with the cultures by the intranasal and subcutaneous routes and reisolated the organism from affected experimental animals.

Organisms Isolated from the Field Disease

Of the authors who isolated mycoplasma from field cases of CCPP, Christodoulu and Talartzis (1957), El Nasri (1967), Pillai (1965), and Solana and Rivera (1967) described their pathogenicity.

Christodoulu and Talartzis (1957) found their agent to be pathogenic only by the subcutaneous route, causing local oedema and death within 4 - 9 days; It did not cause death by the intravenous,
intranasal, or intrapulmonary routes.

El Masri (1967) found his agent (GPS) caused local reaction and death in 3 out of 5 goats inoculated by the subcutaneous route within 7 - 10 days. In addition he observed it killed 2 goats when intranasally insufflated, and an unstated number of sheep were positive by the subcutaneous route of inoculation. Cattle were unaffected. This author examined 60 diseased goat sera by agglutination test and found 50 positive to GPS and OSB42 (see Table 2 for index to agents). He isolated his agent from only 2 out of 60 diseased goats, although he stated such agents were 'always seen in the dark ground preparations of infected pleural exudate.'

Pillai (1965) isolated mycoplasma from 148 of 150 field cases of CCPP in the Sudan. He showed 2 of his isolates to be pathogenic to 7 out of 8 goats by the intranasal and intratracheal routes. However 3 out of 4 of his control goats also developed pneumonia and died.

By intravenous inoculation of goats Solana and Rivera (1967) compared the pathogenicity of pleural exudate from field cases with the pathogenicity of 2 isolates. The experimental disease was the same for the 3 inocula and the same as the field disease. These authors made 2 isolates from 2 different outbreaks, but they did not state the number of affected goats culturally examined.

Heikkila and Ozkal (1953) described a pathogenic mycoplasma which reproduced the natural disease when inoculated into goats by the intratracheal route. It was not clear whether these authors isolated the agent from field cases or whether they referred to an organism which had been maintained in goats by weekly passage.
<table>
<thead>
<tr>
<th>Strain</th>
<th>Isolated from CCPP</th>
<th>Country of isolation</th>
<th>Isolated or studied by</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG₃</td>
<td>+</td>
<td>Turkey</td>
<td>Chu &amp; Beveridge (Cottew and Leach, 1969)</td>
</tr>
<tr>
<td>PP goat</td>
<td>p</td>
<td>&quot;</td>
<td>Chu &amp; Beveridge (Cottew and Leach, 1969)</td>
</tr>
<tr>
<td>GPA</td>
<td>+</td>
<td>&quot;</td>
<td>Al-Aubaide et al. (1972)</td>
</tr>
<tr>
<td>1488EAH</td>
<td>+</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>1117CHU</td>
<td>+</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>BRU</td>
<td>+</td>
<td>&quot;</td>
<td>Cottew et al. (1969)</td>
</tr>
<tr>
<td>BQT</td>
<td>+</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Erdek</td>
<td>+</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Yamut B</td>
<td>+</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Smith (Sm)</td>
<td>+</td>
<td>&quot;</td>
<td>Pendik UN Sheep Dis. Lab.</td>
</tr>
<tr>
<td>Covenli 2</td>
<td>-</td>
<td>&quot;</td>
<td>Arisoy et al. (1967)</td>
</tr>
<tr>
<td>Covenli 3</td>
<td>-</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Y</td>
<td>+</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Ban</td>
<td>+</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Go</td>
<td>+</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Razi</td>
<td>-</td>
<td>Iran</td>
<td>Cottew et al. (1969)</td>
</tr>
<tr>
<td>N108</td>
<td>+</td>
<td>Nigeria</td>
<td>Longley (1951)</td>
</tr>
<tr>
<td>Vom</td>
<td>+</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>OSB42</td>
<td>+</td>
<td>&quot;</td>
<td>Lindley (Cottew and Leach, 1969)</td>
</tr>
<tr>
<td>Vom₁</td>
<td>+</td>
<td>&quot;</td>
<td>Griffin (1964)</td>
</tr>
<tr>
<td>Kaduna₁</td>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Strain</td>
<td>Isolated from CCPP</td>
<td>Country of isolation</td>
<td>Isolated or studied by</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------</td>
<td>----------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>G3</td>
<td>+</td>
<td>Nigeria</td>
<td>Griffin (1964)</td>
</tr>
<tr>
<td>S28</td>
<td>+</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>C11</td>
<td>+</td>
<td>Chad</td>
<td>Perreau (1971)</td>
</tr>
<tr>
<td>Faroha</td>
<td>+</td>
<td>&quot;</td>
<td>Provost et al. (1964)</td>
</tr>
<tr>
<td>GPS</td>
<td>+</td>
<td>Sudan</td>
<td>El Nasri (1967)</td>
</tr>
<tr>
<td>Pillai (P)</td>
<td>+</td>
<td>&quot;</td>
<td>Pillai (1965)</td>
</tr>
<tr>
<td>143-A66 Conn.</td>
<td>-</td>
<td>USA</td>
<td>Al-Aubaidi et al. (1971)</td>
</tr>
<tr>
<td>208</td>
<td>-</td>
<td>&quot;</td>
<td>Yedloutschnig et al. (1971)</td>
</tr>
<tr>
<td>169</td>
<td>-</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>212</td>
<td>p</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>222</td>
<td>-</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Jonas goat (Conn)</td>
<td>-</td>
<td>&quot;</td>
<td>Jonas &amp; Barber (1969)</td>
</tr>
<tr>
<td>Pearson</td>
<td>p</td>
<td>&quot;</td>
<td>Pearson et al. (1972)</td>
</tr>
<tr>
<td>Sparta</td>
<td>-</td>
<td>Sparta</td>
<td>Debonera (1937)</td>
</tr>
<tr>
<td>1PX</td>
<td>-</td>
<td>France</td>
<td>Perreau et al. (1972)</td>
</tr>
<tr>
<td>Mexico</td>
<td>+</td>
<td>Mexico</td>
<td>Solana &amp; Rivera (1967)</td>
</tr>
<tr>
<td>Y goat</td>
<td>-</td>
<td>Australia</td>
<td>Laws (1956)</td>
</tr>
</tbody>
</table>
Table 2. continued

<table>
<thead>
<tr>
<th>Strain</th>
<th>Isolated from CCPP</th>
<th>Country of isolation</th>
<th>Isolated or studied by</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 goat</td>
<td>-</td>
<td>New Guinea</td>
<td>Olds (Hudson et al. 1967)</td>
</tr>
<tr>
<td>A1</td>
<td>-</td>
<td>Edward</td>
<td>(1953)</td>
</tr>
</tbody>
</table>

**Key**

+ : from CCPP  
- : not from CCPP  
p : possibly from CCPP  
: information not found

In brief, apart from El' Naeti (1967) there was no serological evidence in any of these pathogenic strains of the natural transmission of infection. A further deficiency was the lack of experimental study, so that the possibility of mixed cultures was not minimized in this way.

**Reference Strains**

Several authors have mentioned strains of *N. fowleri* isolated from apparently normal field cases of CCPP and have compared these strains serologically (Schoffet et al., 1969; Edward, 1953; Ottawa, 1968; Lemoine, 1968). The accepted reference strain of *N. fowleri* of 0 strain of *N. fowleri* subspecies is 20771, National Collection, GenBank number Z117. This strain was isolated by Schoffet and co-workers at Cambridge and was compared to other strains isolated from 2 goats affected with CCPP in Turkey (Schoffet et al., 1969). Since then other workers have used this strain to study the relationship between this subspecies and other sylvatic strains.
Cottew, Watson, Erdag, and Arisoy (1969) stated strains BRU, BQT, and Erdek (Table 2) were isolated in Turkey from classical CCPP cases. No details of the field condition and frequency or method of isolation were mentioned. The results of their transmission experiments in goats were significant. Provost (1966) isolated 42 mycoplasma from 60 field cases of CCPP, but he described pathogenicity only with 2 of these isolates in cattle.

In brief, apart from El Nasri (1967) there was no serological evidence relating any of these pathogenic mycoplasma to the natural disease, and there was little data on the frequency of primary isolation of these organisms. A further deficiency was the lack of any successful contact transmission of culture induced disease. None of these workers purified their isolates prior to experimental study, so that the possibility of mixed cultures was not minimized in this way.

Reference Strains

Several authors have mentioned strains of mycoplasma isolated from field cases of CCPP and have compared these strains serologically (Cottew et al., 1969; Edward, 1953; Griffin, 1969; Lemcke, 1964).

The accepted reference strain of the aetiological agent of CCPP is PG3 (Edward 1955) the type strain of *M. mycoides* subspecies *capri*, National Type Culture Collection number 10137. This agent was isolated by Chu and Beveridge at Cambridge who received samples of pleural fluid from 1 goat affected with CCPP in Turkey (Cottew and Leach, 1969). Since then other workers have used this strain or subcultures derived from it to study the relationship between this subspecies and other mycoplasma.

The position of a second common reference strain PP goat, has just been clarified by Lemoke (1974), and Tully, Barile, Edward,
Theodore, and Erno (1974). This strain was reported to have been isolated from CCPP in Turkey (Klieneberger-Nobel and Cuckow, 1955) and was believed to be the organism isolated by Beveridge and Chu. Cottew et al. (1969) in Turkey referred to PG₃; their PG₃ has now been shown to be PP goat and 'The origin of PP goat must now be regarded as uncertain' (Lemcke, 1974). Tully et al (1974) have classified PP goat in the species \textit{M. capricolum}.

Other reference strains used include Vom, N108, and OSB42, all of which have been claimed to originate from CCPP cases during Longley's studies in Nigeria. The Farcha strain was isolated in Chad, while the strains described by Pillai and others isolated by Dawi and by El Nasri (1967) in the Sudan have also been studied. Recently Cottew et al. (1969) in Turkey reproduced pleuropneumonia in goats with several mycoplasma strains isolated from CCPP (strains Eqt, Erdek, and Smith). These authors obtained the strain 'Smith' from Dr G.R. Smith, Nuffield Institute of Comparative Medicine, London, but originally it was isolated from CCPP in Turkey.

It is very important to realise that the absence of data on the original field cases and outbreaks negates any claim that these strains caused the disease as observed in the field. However there was usually evidence that the strain was able to cause experimental pleuropneumonia in goats. The absence of any positive spread by contact of the experimental disease induced by cultures precludes the term 'contagious' from the condition initiated. Nevertheless, since these agents have been isolated from cases of natural CCPP or the experimental disease induced with infective material from such cases the possibility of aetiological significance in the natural disease cannot be excluded.
Characteristics of Organisms Reported at the Time of Isolation

Notoriously it has been difficult to obtain adequate intraspecies differentiation of mycoplasma biochemically. Some of the differences between alleged agents of this disease may be accounted for in this way.

With the exception of El Nasri (1967) and Solana and Rivera (1967) the authors who described primary isolation of pathogenic mycoplasma from CCPP did not investigate their own strains extensively in the laboratory. However their observations must be given more weight than those of subsequent workers studying organisms from atypical syndromes or reference strains of limited history.

Morphology. Christodoulu and Talartzis (1957), Heikkila and Ozkal (1953), Longley (1951), and Shirlaw (1949) considered that their organisms resembled the agent of CEPF morphologically, forming cocci, rings, branching filaments, points, and stars. Christodoulu and Talartzis (1957), Heikkila and Ozkal (1953), and Shirlaw (1949) all found their agents passed through a Seitz filter.

Rate of growth. Christodoulu and Talartzis (1957), Heikkila and Ozkal (1953), and Shirlaw (1949) found their isolates grew rapidly to high titre within 24 to 72 hours. El Nasri (1967) observed very slow growth which improved after repeated passage while Longley (1951) recorded slow growth using neat goat and sheep serum as medium. Abdulla and Lindley (1967) failed to achieve adequate growth of their isolates.

Haemolysis. Christodoulu and Talartzis (1957) observed alpha haemolysis while Solana and Rivera (1967) found greenish haemolysis on solid medium containing 5 per cent rabbit red blood cells.

Liquefaction of inspissated medium. Longley (1951) showed his mycoplasma liquefied inspissated sheep and goat serum.
Fermentation. The organism isolated by Solana and Rivera (1967) fermented 1 per cent glucose, laevulose, maltose, and mannose in liquid medium. El Nasri (1967) found his agent fermented glucose, fructose, maltose, mannose, glycogen, dextrin, and starch, while OSB42 fermented mannitol as well. None of these or PG₃, N108, or Pillai's strains fermented galactose, lactose, salicin, raffinose, arabinose, rhamnose, or trehalose.

Pathogenicity to chicken embryos. Christodoulu and Talartzis (1957) and Solana and Rivera (1967) observed that their organisms inoculated via the chorionicallantoic and yolk sac routes respectively were highly pathogenic to 9 - 11 day old embryonated eggs, causing death within 24 - 48 hours. The agent isolated by Heikkila and Ozkal (1953) also grew in 6-day-old embryonated eggs. Christodoulu and Talartzis (1957), and Heikkila and Ozkal (1953) found their organisms grew in all parts of the egg while Solana and Rivera (1967) found abundant growth from the yolk and chorionicallantoic fluid as well as intense haemorrhage of the embryo.

Serology. Other than El Nasri (1967), Longley (1951), and Solana and Rivera (1967) authors who isolated pathogenic mycoplasma from classical cases of CCPP did not record any serological investigation of their isolates.

By agglutination tests El Nasri (1967) found slight cross reactions between PG₃, N108, and Pillai strains. The agents were also shown to cross-react with his GPS strains. However since he did not prepare antisera to GPS the relationship was difficult to assess. He also found a strong relationship between Pillai strains and M. mycoides subspecies mycoides by gel diffusion test but no relationships between the other strains by this test.

Longley (1951) sent a Nigerian isolate to Klieneberger-Nobel
at the Lister Institute in 1941. She found his agent was not related to *M. agalactiae* and showed very slight cross-reaction with *M. mycoides* subspecies *mycoides* by the agglutination test.

Solana and Rivera (1967) compared their organism with Longley's 'Vom' strain. They found the organisms closely related by slide and tube agglutination test, interphase precipitation test, and growth-inhibition test.

However, as will be shown later, the literature has only recently been clarified as to the characteristics of CCPP reference strains such as Vom, PG3, OSB42, and N108. This seriously detracts from the value of comparative tests with many pathogenic mycoplasma isolates from this disease.

**General Morphological and Cultural Characteristics**

Edward (1953, 1954, 1955) investigated the cultural and biochemical characteristics of the Chu and Beveridge strain and of a strain isolated by Longley in Nigeria. It is relevant that Longley (1951) did not record transfer of the organism to this author since several strains have been attributed to Longley's studies.

Edward (1953) found the Chu and Beveridge strain (PG3) fermented dextrin, starch, and glycogen in addition to glucose, laevulose, maltose, and mannose. This agent did not ferment lactose, saccharose, mannitol, galactose, dulcitol, or salicin. Colonies were haemolytic on horse blood agar and the supernate of broth culture discoloured horse erythrocytes. The organism liquefied inspissated serum. Growth in medium without serum was poor but it was equally good on medium containing horse or rabbit serum. Colonies of this strain and Longley's strain grew rapidly to form colonies up to 1.5 mm in diameter.
Hudson, Cottew, and Adler (1967) observed their reference strain, *M. mycoides* subspecies *capri* (perhaps PP goat (Lemcke, 1974) because it was 'probably isolated by Dr Chu in Turkey from a case of CCPP' and obtained from Kleineberger-Nobel (Cottew and Lloyd, 1965)) caused slight acidity when grown in medium containing sucrose or galactose in addition to fermenting glucose, maltose, laevulose, and mannose. This result contrasted with the above but it may be merely that Hudson et al. (1967) considered a smaller drop in pH to be significant.

Cottew et al. (1969) compared 8 Turkish mycoplasma isolates with strains Smith, PG3 (PP goat (Lemcke, 1974)), N108, and Vom. They grouped 4 Turkish isolates from CCPP (including strain Smith) together, PG3 with N108, and Vom with *M. mycoides* subspecies *mycoides*. The first group formed points and clusters in liquid medium; colonies with a central nipple, 0.5 - 1 mm in diameter, on solid medium; showed strong haemolysis with different patterns according to the species of red blood cells, namely horse, chicken, or sheep; liquefaction of inspissated horse serum; and strong fermentation of glucose which occurred more slowly than that of PG3 and N108. The second group formed colonies up to 1.7 mm in diameter with a central nipple and, in older cultures, multiple papillae. They showed strong but less characteristic haemolysis; and very rapid fermentation of glucose. The Vom strain differed from the above two groups, forming branching filaments in liquid medium, colonies up to 1 mm in diameter with a well-defined central nipple, and showing weak haemolysis and very slight liquefaction of inspissated horse serum. Fermentation of glucose by the Vom strain
was not tested. The usefulness of the different patterns of haemolysis observed by these workers as a means of differentiating organisms was limited by absence of technical detail, for example, age of colonies observed.

The failure of some of their strains to form filaments is noteworthy. Filaments seen by early workers in exudates from field cases of CCPP provided the basis of a diagnostic technique using dark-ground examination of pleural fluid withdrawn during life (Abdulla, 1966).

Other authors have observed different patterns of haemolysis depending on the species of red blood cells used. Cole, Ward, and Martin (1968), using centrifuged deposits of broth cultures of a strain received from D.G. ff Edward, found beta–haemolysis on solid medium containing rabbit, sheep, or guinea pig red blood cells. On the same medium containing chicken or duck red blood cells they observed alpha–haemolysis with a beta–halo. Results varied according to techniques since Ernö and Stipkovits (1973), studying colonies of PG₃ overlaid with agar containing different red blood cells, found alpha–haemolysis with sheep red blood cells and, after further incubation at 22°C, beta–haemolysis. This may not be a valid comparison because the strain supplied by D.G. ff Edward to Cole et al. (1968) could have been either the PG₃ or the Longley strain (Edward, 1953).

Manchee and Taylor–Robinson (1968) found that M. mycoides subspecies capri did not haemagglutinate or haemadsorb. Their strain of M. mycoides subspecies capri derived from Dr R.H. Leach and was probably PG₃. Ernö and Stipkovits (1973) found PG₃ haemagglutinated guinea-pig red blood cells. It may be significant
that the former authors tested centrifuged mycoplasma deposits while the latter used cultures. Cherry and Taylor-Robinson (1970, 1973) showed that the liberation of peroxide was an important factor in the pathogenesis of *M. mycoides* subspecies *capri* infection of chicken tracheal organ cultures. In the former study the authors also showed that HeLa cells adsorbed to colonies of *M. mycoides* subspecies *capri*.

Prior work by Cole et al. (1968) had shown colonies of *M. mycoides* subspecies *capri* produced peroxide and that there was a correlation between peroxide production and haemolysis; while peroxide was a major haemolysin of this organism, other haemolysins were also involved. As mentioned above, there was no indication whether this organism was PG₃ or the Longley strain, also studied by Edward (1953, 1954).

The media which failed to support growth of these agents were as sophisticated or more so than those which were successful. Beaton (1931) found his agent grew on ox serum agar and on inspissated goat serum, in contrast to Longley (1940) who observed and recognized mycoplasma in affected tissues and exudates but failed to grow them on goat serum agar, *viande foie* medium (Turner, Campbell, and Dick, 1935) with goat or ox serum, or other special media prepared from goat flesh, blood, and serum. Similarly Abdulla and Lindley (1967) failed to grow these agents on a sophisticated medium including additions such as yeast extract, liver digest, and DNA, while Beaton (1931) and Shirlaw (1949) found Martin's and Bennett's broth to be satisfactory. These records give no indication that the organism preferred any one animal serum, since ox, sheep, goat, rabbit, and horse serum have all been successfully employed,
although Shirlaw (1949) considered goat serum the serum of choice, ox serum being less satisfactory and horse serum definitely inhibitory. These inconsistencies are resolved if more than one organism can cause the experimental disease following inoculation of goats and sheep.

Ultrastructure

There are few electron microscope studies of CQPP agents in the literature. Domermuth, Nielsen, Freundt, and Birch-Andersen (1964) studied confluent colony sections of PG3. Klieneberger-Nobel and Cuckow (1955) used shadow casting with gold-palladium alloy to observe the morphology of PP goat in broth culture, while Chu and Horne (1967) studied negatively-stained preparations of strains of M. mycoides subspecies capri prepared from broth cultures, strain Ankara/49 isolated by Chu, and strain G1/61 isolated by Pillai. Recently Rodwell, Peterson, and Rodwell (1972, 1973) and Peterson, Rodwell, and Rodwell (1973) described the rho form of the Y goat strain, the V5 vaccine strain of M. mycoides subspecies mycoides, and other mycoplasma organisms. The rho forms were commonly found in caprine mycoplasmas. The growth medium was important for the selection of the rho form genotype, a medium of relatively high tonicity and unrestricted energy source being required. These forms were elongated with beaded swellings and contained a striated filamentous structure visible in negatively-stained preparations or in sections. They were found only in organisms exceeding about 0.75 μm, extending throughout the entire length of the organism, some of which measured up to 5 μm. The periodicity of the striations varied in different organisms from 12 – 14.5 nm, while the parallel fibrils were about 3 nm in
diameter. In a high proportion of organisms the striated inclusions terminated at one end or, less frequently, at both ends, in a small, knob-like swelling with a characteristic structure. The nature and function of the terminal structure and striated inclusions are unknown. However evidence indicated the fibre contained protein.

**Mycoplasma from Atypical CCPP**

Several mycoplasma strains classified as M. mycoides subspecies capri have been isolated from syndromes other than CCPP (Edward, 1953; Jonas and Barber, 1969; Melanidi, 1951; Pearson et al., 1972; Perreau et al., 1972; Yedloutschnig et al., 1971). The classification of these agents rested largely on laboratory investigation of the organisms and on the parenteral induction of experimental disease in goats.

Melanidi (1951) isolated an organism from fatal oedematous cellulitis which he considered to be an agent of contagious agalactia. However Edward (1953) found this mycoplasma had exactly the same cultural and biochemical properties as the organism of goat pneumonia represented by the Chu and Beveridge strain. Melanidi (1951) described his agent as filamentous, viable in ampoules of oedema fluid or infected blood for 8 months, and able to pass a Chamberland L2 filter. It was killed by heating at 60°C, drying, 1 - 2 parts per 1000 parts formalin for 24 hours at 37°C, and 1 part per 1000 parts saponin for 0.5 hours at 37°C.

Jonas and Barber (1969) also showed their isolate had similar cultural characteristics to PG3 (Edward, 1953). Their agent formed pin point alpha-haemolytic colonies on sheep blood agar and colonies over 1 mm in diameter within 3 days on Hayflick's
mycoplasma medium, passed through a 0.45μm filter but not a 0.2μm, and liquefied inspissated horse serum. This mycoplasma fermented several carbohydrates additional to PG₃, namely, galactose, lactose, saccharose, mannitol, and trehalose. It is interesting that El Nasri's results (1967) also differed; OSB42 fermented mannitol, and none of the strains he studied fermented trehalose.

Yedloutschnig et al. (1971) found the 4 mycoplasma isolates they studied fermented the same carbohydrates, grew on mycoplasma medium containing rabbit serum, and killed 9-day-old embryonated hen's eggs when inoculated via the yolk sac.

Perreau et al. (1972) merely determined their isolate fermented glucose, while Pearson et al. (1972) cultured their organism in embryonated eggs which were killed in 48 hours. The classification of the last strain as M. mycoides subspecies capri was carried out by the Plum Island Animal Diseases Laboratories.
EXPERIMENTAL PATHOGENICITY OF CAPRINE *M. mycoides.*

Isolates from the Classic Disease

El Naṣri (1967) tested several CCPP agents in rabbits, guinea pigs, sheep, goats, and cattle. Strains PG₃ and Pillai were avirulent to all species while N108 caused a local reaction and death in 1 goat. Strains OSB42 and GPS (GPP/1) were virulent to sheep and goats by the subcutaneous route and also caused pleuropneumonia in goats following intranasal inoculation. Bulls inoculated with OSB42 were fully susceptible when challenged 3 weeks later with *M. mycoides* subspecies mycoides strain 121.

Using groups of 4 - 6 goats per experiment, strains BQT, Erdek, and Smith (Table 2) were inoculated by the intratracheal route and caused pleuropneumonia after 4 and death within 4 - 7 days, with the exception of one goat killed 10 days post inoculation (Cottew et al., 1969). Strains BQT and Smith were each injected subcutaneously into a group of 3 goats causing death in under 11 days; subcutaneous oedema in all; and pleuropneumonia in all but one. In a similar experiment the Erdek strain killed 3 goats without causing pleuropneumonia, and subcutaneous lesions in 1 only.

The Erdek strain was intratracheally inoculated into 3 sheep also, causing death from pleuropneumonia in 1 within 6 days and necrosis at the site of inoculation in this animal and 1 other. Except for 1 of the inoculated sheep the agent tested was recovered from all experimental animals.

These authors also inoculated strains Yamut B, OSB42, Yamut A, Vom, N108, Chu and Beveridge (PG₃), each into 4 goats by the intra-
tracheal route. OSB42 caused oedema and necrosis at the site of inoculation and death within 3 - 6 days in 3 out of 4 goats while Yamut A caused death in 5 - 6 days, with slight, atypical pneumonia in 2, and oedema and necrosis at the inoculation site in the remaining 2 goats. Yamut B merely caused slight atypical pneumonia in 1 goat. The goats inoculated with the other strains remained unaffected although occasionally mycoplasma were isolated on slaughter.

In assessing these results it should be remembered that the oedematous necrotic lesions caused by OSB42 are characteristics of agents of CCPP and the failure to cause pleuropneumonia by all these strains might be an indication either of reduced virulence or of resistance of the goats to infection with this agent. The latter observation perhaps should be given more weight in the light of recent work indicating such agents may cause natural syndromes other than CCPP (Perreau et al., 1972; Yedloutschnig et al., 1971). The authors did not test their experimental animals for antibodies prior to experiment.

The avirulence of strain PG3 was of particular interest; however this PG3 was in fact PP goat, an agent of uncertain origin (Lemcke, 1974). Although PG3 is the world reference strain of M. mycoides subspecies capri, its pathogenicity has never been investigated extensively. Cottew and Leach (1969) state that Chu and Beveridge inoculated the strain into 2 goats and 1 sheep. In 1 goat infected intranasally with atomized culture classical pleuropneumonia was produced. As mentioned above El Nasri (1967) found this strain to be avirulent.

Recently Goni and Onoviran (1972) in Nigeria provided supporting
evidence for the experimental pathogenicity of strain Smith of *M. mycoides* subspecies *capri*. These authors inoculated 9 goats by repeated intranasal instillation, 7 of which developed pleuropneumonia and died 7 - 18 days post inoculation. Serological evidence of infection was obtained and the agent recovered from all affected goats.

Barber and Yedloutschnig (1970) demonstrated the pathogenicity of strains Vom, Connecticut, and Mexico following intramuscular inoculation into goats, sheep, and calves. All sheep and goats died in 3 - 5 days, calves died between 13 and 17 days. The virulence of their Vom strain contrasted with the findings of Cottew et al. (1969).

With each of these strains only 1 calf died; those that survived the Vom and Mexico strains had mycoplasmaemia, whereas the one that survived the Connecticut strain did not.

Animals inoculated intramuscularly died of mycoplasma 'septicaemia' as indicated by widespread recovery of the organism from the body tissues and gross lesions. Mycoplasma organisms were recovered from joint fluid, cerebrospinal fluid, tonsil, lung, spleen, thoracic fluid, urine, and peripheral and visceral lymph nodes. Gross lesions following intramuscular inoculation were excessive thoracic, peritoneal, and joint fluids, and petechial haemorrhages on the surfaces of several visceral organs. Cellulitis was usually present at the site of inoculation, whether injected intramuscularly or intratracheally.

Two goats were inoculated intratracheally with the Connecticut strain and died 5 days post inoculation, showing cellulitis at the site of inoculation and extensive fibrinous pleuritis. Fourteen other goats were inoculated intratracheally with the Connecticut
strain. Two died 9 and 13 days post inoculation with fibrinous pleuritis, and of 6 which developed local cellulitis 3 died within 12 days and 3 recovered. The remainder were unaffected.

When given to 2 pigs by the intraperitoneal route only the Vom strain killed 1 pig, 11 days post inoculation, with extensive fibrinous peritonitis and a thickened pericardial sac. The organism was recovered from peritoneal fluid, tonsil, lung, spleen, and cerebrospinal fluid. Following intraperitoneal inoculation with the Connecticut strain both pigs developed mycoplasmaemia. All pigs developed mycoplasmaemia after intramuscular inoculation with Vom or Mexico strain. The presence of the organism in the blood was not always associated with illness. All the strains caused mycoplasmaemia when given intravenously to pigs.

**Isolates from the Atypical Disease**

Jonas and Barber (1969) described mild conjunctivitis and periorbital cellulitis following inoculation of a goat with Connecticut strain culture via the conjunctival sac and supraorbital connective tissues. No other lesions except serous lymphadenitis were observed at slaughter. The organism was recovered from the blood on day 1 and day 2, and at post-mortem examination from heart, blood, lung, liver, kidney, urine, spleen, lymph nodes, bone marrow, joint fluid, cerebrospinal fluid, and conjunctiva. The organism also caused acute febrile disease and death within 72 hours when inoculated intravenously into goats. It was termed the Connecticut strain (Barber and Yedloutschnig, 1970).

The agent isolated by Debonera (1937) and also described by Melanidi (1951) from Sparta or oedema disease caused a severe reaction following subcutaneous inoculation of goats or sheep,
while intravenous injection did not kill the animal provided the subcutaneous tissues were not affected. Oral inoculation was followed by septicaemic death within 2 days. Arisoy, Etheridge, Foggie, and Erdag (1969) described a similar disease in Turkey, except sheep were affected though much less severely than goats. One of their strains, Covenli 3, was inoculated subcutaneously into 6 goats and 3 sheep by Watson, Cottew, Erdag, and Arisoy (1968). The latter also inoculated a similar strain from Iran, strain Razi, subcutaneously into 3 goats. The temperatures of 6 animals inoculated with Covenli 3 were recorded, and all showed a rise persisting for 3 - 4 days. All the animals inoculated with Covenli 3 and 2 of the goats given Razi developed a marked oedematous reaction extending along the brisket from the site of injection. Of the goats given Covenli 3, 2 died with peritonitis and mycoplasmaemia, and 1 developed a generalized periarticular reaction. One of the Razi-infected goats, which showed a marked oedematous reaction, also developed extensive pneumonia.

Cottew et al. (1969), on the basis of cultural characteristics and serological studies, differentiated Covenli 3 and Razi strains from their agents of CCPP. However it is interesting to note that the Razi strain caused extensive pneumonia (Watson et al., 1968).

Watson et al. (1968) mentioned the lesions observed following inoculation of Covenli 3 and Razi strains into sheep and goats were similar to those observed by Bory and Entessar (1962) using the Razi strain. The latter gave a detailed description of the experimental disease following subcutaneous inoculation of culture into goats. While they studied 4 isolates, none was referred to as the Razi strain, and so one must assume that Watson et al. (1968)
received one of these isolates which they named Razi. The isolates derived from milk or testicles of naturally-affect ed sheep and goats, and similar organisms were isolated from ocular lesions of such cases. In the experimental disease, Bory and Entessar (1962) mentioned oedema of the udder with subsequent mastitis, keratoconjunctivitis, abscesses, ulceration of the cornea, emaciation, and pericardial petechiae in addition to those lesions described by Watson et al. (1968). Bory and Entessar (1962) considered their strains to be agents of contagious agalactia and they state they were non-haemolytic, while Cottew et al. (1969) found the Razi strain and Covenli strain to be similar to each other, haemolytic, and quite different from M. agalactiae. The work of Debonera (1937) and Melanidi (1951), investigating a condition they considered to be a form of contagious agalactia but the agent of which Edward (1953) considered closely related to that of CCPP, is almost exactly parallel.

Yedloutschnig et al. (1971) used high-titre cultures of 4 isolates to investigate their pathogenicity to goats, sheep, cattle, pigs, and chicken embryos. In goats, intramuscular inoculation of all strains caused fever, lameness, depression, and extensive oedema of the affected limb. Autopsy revealed severe myositis throughout the inoculated thigh muscle with subcutaneous oedema extending towards the distal portion of the leg. Intrathoracic inoculation resulted in a high temperature just before death and severe pleuropneumonia with fibrinous adhesions on the inoculated side, followed by death in 3 – 5 days. In addition, isolate 169 caused arthritis, periarthritis, and synovitis with severe lameness following inoculation into the stifle joint of 1 goat. Sub-
conjunctival inoculation of isolate 222 resulted in swelling, congestion, and oedema of the eye, and masseter and temporal muscles, leading to mycoplasma anaemia and death in 7 days. The herd from which this organism was isolated did not have detectable complement-fixing or precipitating antibody to this organism or to other similar agents. A pregnant goat inoculated intrathoracically with isolate 208 suffered as described above, but the organism was reisolated from both mother and foetus. Dramatic leucopenia preceded death in sheep and goats infected with this organism. In-contact infection was demonstrated with this isolate, while isolate 169 caused the death of an in-contact kid.

Three of the strains were tested by the intramuscular and intrathoracic routes in sheep, with similar results to those found in goats. Isolate 212 did not affect steers and pigs following intrathoracic and intramuscular inoculation respectively, while isolate 169 infected 2 out of 2 calves and killed 1 following intrathoracic inoculation. All these agents killed chicken embryos.

In Arizona, Pearson et al. (1972) isolated a mycoplasma from young kids affected with a syndrome similar to CCPP. The organism caused high fever, severe arthritis, and death within 8 - 10 days when inoculated subcutaneously or intramuscularly into sheep. Experimentally-inoculated goats developed high fever and lobular pneumonia, dying 8 days post inoculation with signs of peracute septicaemia. Although the goats were lame no arthritic lesions were observed. This agent was classified as *M. mycoides* subspecies *capri* at the Plum Island Animal Diseases Laboratory.

Strain 1PX (Perreau et al., 1972) was isolated from the milk of 3 affected goats in a herd suffering a mastitis and arthritis
syndrome. The authors considered their agent belonged to the *M. mycoides* subspecies *capri* group following limited cultural and serological studies.

*M. mycoides* Isolates from Caprine Syndromes not Associated with CCPP Laws (1956) isolated Y goat strain from a goat with diffuse fibrinous peritonitis. This was the only animal affected in a herd of 10 goats. Following intravenous inoculation of culture a goat and a sheep were unaffected. However disease was produced after intraperitoneal inoculation of culture into 1 goat and 2 sheep, a calf being unaffected. One affected sheep and the affected goat developed pyrexia, anorexia, nasal discharge, shallow thoracic respiration, and severe abdominal pain. The goat died 4 days post inoculation with fibrinous peritonitis, oedematous lumbar lymph nodes, and haemorrhagic mediastinal lymph nodes. The organism was reisolated from the nasal discharge and blood 1–2 days before death, and the peritoneal fluid, liver, spleen, pericardial fluid, and associated lymph nodes at autopsy. One sheep showed lesions similar to the above, while the second became emaciated and weak, showing arthritis on autopsy; the mycoplasma was not reisolated from this animal.

Hudson et al. (1967) continued the study of this agent. Six calves were inoculated, 2 intravenously with culture mixed with egg fluids, and 4 subcutaneously with culture. Only 3 developed complement--fixation titres and only 2 of the 3 sera fixed complement with *M. mycoides* subspecies *mycoides* antigen. None of the calves showed symptoms and no lesions were observed in the 2 calves slaughtered. Several older cattle were inoculated subcutaneously but showed no reaction although the organism was reisolated from the
precrural lymph node of the inoculated side of an animal slaughtered 1 month after inoculation. These authors also inoculated 2 sheep intraperitoneally and 2 by the intravenous route with similar inocula. Both the intraperitoneal-inoculated sheep developed disease similar to that in the goat described by Laws (1956) while 1 out of 2 of the sheep inoculated intravenously developed bursitis in the left hind leg, and the agent was reisolated from this lesion.

0 goat was isolated by Olds from a goat with polyarthritis in New Guinea. This organism was studied by Hudson et al. (1967). Of 2 calves inoculated subcutaneously 1 developed a local reaction similar to the Willem reaction as well as severe polyarthritis. The organism was reisolated from the blood and at autopsy from both stifle joints. The second calf developed transient mycoplasmaemia and swelling of the right knee and hock. At autopsy no abnormalities were observed. Two calves were also inoculated intravenously and both developed mycoplasmaemia. At autopsy 1 showed synovitis of 2 joints but no mycoplasma were reisolated, while the other had sequestrae in the lungs from which the agent was recovered. All the calves developed complement-fixing antibodies to the homologous organism and to M. mycoides subspecies mycoides. This agent and the Y goat organism were shown to be very closely related to M. mycoides subspecies mycoides.

C11 (Perreau, 1971; Provost, 1966) was isolated in Chad from an affected goat in a herd suffering from enzootic pneumonia. Pleural lesions were rare, the main lesion being lobar pneumonia, affecting principally the apical and cardiac lobes. Histological section of hepatized lesions showed the lung lesion to consist of
broncho-alveolar supplicative foci with inflammatory oedema of the interlobular septae and some peribronchial lymphocytic infiltration. By various serological tests Perreau (1971) concluded that his Vom strain and C11 were the same as *M. mycoides* subspecies *mycoides*. Both the above authors described experimental inoculation of cattle with C11: the following details derive from Provost (1966). Nine young cattle were inoculated endobronchially and kept in contact with 4 others. No symptoms developed except transient coughing in 2 animals. The intubated cattle developed complement-fixation test titres of 320 - 640, the titre latterly falling to normal. At slaughter, 3 months later, there were no lesions and no mycoplasma reisolated from pulmonary lymph nodes.

Another strain isolated in Chad from goat lungs, M108, caused circumscribed pleuropneumonia lesions in 2 cattle (Perreau, 1971). Provost (1966) mentioned that from 60 caprine pleurisy and pneumonia cases 42 mycoplasma were isolated. Twelve of the strains were inhibited by growth-inhibiting serum to *M. mycoides* subspecies *mycoides*, so that there is some justification for the view that CCPP may be a syndrome caused by different mycoplasma (Perreau et al. 1972).

In Kano, Nigeria, Oja (1973) isolated two strains of *M. mycoides* subspecies *mycoides* from the lungs of 2 pneumonic goats. These strains were studied biochemically and culturally and classified by growth inhibition tests. Inoculated goats suffered necrotic myositis and pneumonia not typical of CCPP. The organisms were recovered from the experimental goats. He found 80/115 sera from field goats had titres in excess of 1/20 to the isolated mycoplasma species.
PATHOGENICITY OF M. MYCOIDES SUBSPECIES MYCOIDES OF BOVINE ORIGIN FOR SHEEP AND GOATS

In view of the many isolates of M. mycoides subspecies capri subsequently found to be the same in vitro as bovine M. mycoides subspecies mycoides it is relevant to consider the pathogenicity of virulent bovine 'M. mycoides subspecies mycoides' for goats and sheep.

Dick (1937) inoculated cultures of M. mycoides subspecies mycoides subcutaneously into goats. Oedematous swellings followed about the site of inoculation behind the shoulder, and sometimes lameness developed. The swellings appeared 3 – 7 days after inoculation and persisted for 3 weeks. Some of the goats developed complement-fixing antibodies to M. mycoides subspecies mycoides, and most of them became immune to further inoculation. Four-month-old goats were not susceptible while those over 12 months reacted as above.

Turner et al. (1935) reported similar symptoms with cultures or pleural exudate. Sheep appeared to be more susceptible than goats, and frequently died as a result of inoculation. The organism was recovered from the blood-stream and from various internal organs. Complement-fixation antibodies were demonstrable.

Beller and Tahssin-Bey (1926) also found similar syndromes following subcutaneous inoculation of goats and sheep. In addition, the inoculation of pregnant ewes led to infection in utero. Sometimes the ewe aborted, on other occasions the lamb appeared normal at birth or was affected with articular swellings; some of the lambs died. The prominent foetal lesion was fibrinous serositis and the organism was recovered from the serous fluid.

Abdulla and Lindley (1967) and Lindley and Abdulla (1969)
failed to cause disease or lesions in goats and sheep by endo-
bronchial inoculation of virulent cultures or CBPP lung
suspension. They found the inoculated sheep and goats did not
develop complement-fixing antibodies, although the lung of an
inoculated goat and lymph nodes of all inoculated sheep and goats
were positive by agar gel double diffusion test.

Hudson (1971) 'knows of no experimental evidence of the
induction of pneumonia in sheep or goats by exposure of these
animals to nebulised cultures of virulent M. mycoides of bovine
origin, and there is no clinical or experimental evidence which
would indicate that CBPP has been spread naturally or
experimentally by goats to cattle.' Most of the recorded work
refers to the subcutaneous inoculation of sheep and goats, and
even M. mycoides subspecies mycoides inoculated subcutaneously
into cattle rarely causes lung lesions (Hudson, 1971).
M. MYCOIDES FROM SPECIES OTHER THAN CATTLE AND GOATS

The isolation of mycoplasma from COPP and other diverse caprine syndromes which showed a very close relation to M. mycoides subspecies mycoides is not without parallel in other species. Erns, Freundt, Kroggaard-Jensen, and Rosendal (1972) identified an organism (strain 2833) isolated from maned sheep in a zoo at Frankfurt by Brack (1966) as M. mycoides subspecies mycoides. Brack reported that the organism caused mortality in the young (19 - 105 day old). The disease was characterized by myocarditis, pericarditis, arthritis, and enlargement of the spleen and liver.
SEROLOGICAL RELATIONSHIPS BETWEEN CAPRINE *M. mycoides* AND BETWEEN THESE ORGANISMS AND *M. mycoides* SUBSPECIES *MYCOIDES*

Since nearly all mycoplasma associated with the aetiology of CCPP have been shown to bear a relation to *M. mycoides* subspecies *mycoides*, caprine isolates apparently the same as *M. mycoides* subspecies *mycoides* which were experimentally pathogenic to goats must also be considered.

**Inter-relationship of Caprine *M. mycoides* Organisms**

Although there are wide variations in the degree of relatedness between reference strains of CCPP, the variations may be purely technical. It is probably inadvisable to rely on tests conducted with antisera from different species prepared in different ways. In short, there is a clear need for reference antisera. Furthermore, the inter-relationships of these mycoplasma have not been extensively studied by any serological test.

**Agar gel diffusion test.** El Nasri (1967) found no relationship between PG3, OSB42, GPS, and Pillai strains. PG3, PP goat, and Y goat strains were shown to be closely related by Argaman and Razin (1969); Lemcke (1964) showed PP goat was related to Pillai strain P1/61, and in 1974 she observed PG3 and PP goat to be related.

The results of Jonas and Barber (1969) and Yedloutschnig et al. (1971) are in agreement; strains Vom, Mexico, Conn., 169, 208, and 212 were found to be related by agar gel diffusion test. In general this technique seemed of value in placing agents in the *M. mycoides* species. Perreau et al. (1972) related their isolate 1PX with OSB42 and PG3 in this way.

**Complement fixation test.** As with the agar gel diffusion test Perreau et al. (1972) found 1PX to be related to OSB42 and PG3.
Similarly Jonas and Barber (1969) and Yedloutschnig et al. (1971) found Vom, Mexico, Conn., 169, 208, and 212 to be related. Vom was also observed to cross react with Farcha strain (Provost, Villemot, Queval, and Borredon, 1964). Later, strains Conn., 143, 169, 208, and 212 were found to bear a relationship to N108 (Stone and Yedloutschnig, 1973).

In contrast to her agar gel diffusion test results Lemcke (1964) found PP goat and Pillai strain Q1/61 were only weakly related by complement fixation tests. Hudson et al. (1967) found PP goat only weakly related to 0 and Y goat strains, which were closely inter-related.

**Tube agglutination test.** In support of their complement-fixation study Hudson et al. (1967) found Y goat and 0 goat closely related and only very weakly related to PP goat. They also found one of Pillai's strains reacted much more strongly to 0 and Y goat strains than to PG3. Similarly Perreau et al. (1972) again showed 1PX to be related to OSB42 and PG3, while Provost et al. (1964) observed a relation between strains Vom and Farcha. Solana and Rivera (1967) found strain Mexico reacted with antiserum to the Vom strain. El Nasri (1967) observed GPS reacted more strongly with OSB42 antiserum than with antisera to N108 or a Pillai strain.

Cottew et al. (1969) divided their strains into 2 groups by tube-agglutination test: in one group BRU, BQT, Smith, Erdek, and N108; in the second group Cov2, Cov3, Razi, Yamut B, OSB42, and Vom. These 2 groups did not cross react significantly and neither showed a significant reaction to PG3 (PP goat (Lemcke, 1974)). The second group was related to M. mycoides subspecies mycoides.

**Fluorescent antibody tests.** Tessler (1973b) showed strains Vom,
Mexico, Conn., and Maryland (169 or 208) could be differentiated using an incident ultraviolet light technique. In an earlier paper Tessler and Yedloutschnig (1972) found these strains closely related using a transmitted ultraviolet light technique. Similarly Perreau et al. (1972) showed a relation between PG₃ and 1PX.

**Growth and metabolic inhibition tests.** Al-Aubaidi, Dardiri, and Fabricant (1972) arrived at a division of strains, based on metabolic and growth inhibition tests. Their first group, Group 3, included GPA, Farcha, PG₃, Smith, and BQT, while the second, Group 8, comprised Y goat, O goat, P goat, OSB42, Vom (Perreau), Vom (PIADL), Vom (Longley), Conn., 208, 222, Mexico, Razi, Yamut B, Cov₂ (2406), Cov₃ (2577), Jonas goat, BRU, 1488 (EAH), CHU, and ClI (Chad). In contrast to group 3, group 8 agents were related to *M. mycoides* subspecies *mycoides* and "should be considered as *M. mycoides* subspecies *mycoides* of caprine origin". Y goat was selected as the type strain of group 8. The growth inhibition test results of most earlier workers supported this classification (Cottew et al., 1969; Perreau, 1971; Solana and Rivera, 1967; Jonas and Barber, 1969; Yedloutschnig et al., 1971). In addition the findings of Cottew et al. (1969) suggested that N108 belonged to Group 3 while those of Yedloutschnig et al. (1971) place strains 169 and 212 in Group 8.

**Relationship between Caprine *M. mycoides* and *M. mycoides* subspecies *mycoides***

**Agar gel diffusion test.** Agar gel diffusion studies show a wide variation in results. At one extreme, El Nasri (1967) found no relationship between OSB42, PG₃, GPS, and *M. mycoides* subspecies *mycoides*, strain 121, although he found no difference between Pillai strains and *M. mycoides* subspecies *mycoides*, strain 121.
At the opposite extreme, Argaman and Razin (1969) showed that PP goat, PG3, and strain Y were not only similar to each other but also very closely related to M. mycoides subspecies mycoides, V5, and PG1. They showed these relationships using soluble cell proteins or SDS (sodium dodecyl sulphate) solubilized membranes as antigen. Lemcke (1965) considered PP goat and M. mycoides subspecies mycoides probably had 3 antigens in common. In 1974 this author confirmed PP goat and M. mycoides subspecies mycoides (PG1) were related, and showed a similar relationship with PG3.

In 1972 Provost found Vom, unlike Farcha, was a strain of M. mycoides subspecies mycoides, and that strain Farcha did not possess galactan. He also found that although strain Y contained a polysaccharide resembling galactan it was not identical to the polysaccharide of M. mycoides subspecies mycoides.

In Australia sera from cattle infected with M. mycoides subspecies mycoides gave strong precipitation bands, not only with M. mycoides subspecies mycoides but also with the 2 caprine M. mycoides strains available. The patterns of lines differed in the 2 systems; after many tests, in only 1 case was a band apparently common to both organisms. Commonly the lines formed against M. mycoides subspecies capri could be seen to cross the M. mycoides subspecies mycoides lines (Anon, 1964).

Complement fixation test. The results of complement fixation studies showed a similar disunity. Griffin (1969) reported strains of M. mycoides subspecies mycoides and M. mycoides subspecies capri, including N108, showed cross reactions of approximately 25 per cent identity. Ernø et al. (1972) and Provost et al. (1964) observed strong one-way cross reactions with antisera to strains Vom and
Farcha against \( V_5 \) antigen and antiserum to \( PG_1 \) and \( PG_3 \) antigen respectively. Edward (1953), Edward and Leach (Provost et al., 1964), Freundt, Erns, Black, Kroggaard-Jensen, and Rosendal (1973), Hudson et al. (1967), and Lemcke (1964) observed low-titre cross reactions between PP and \( PG_3 \) strains and \( M. \text{mycoides} \) subspecies \( \text{mycoides} \). Edward also showed a one-way relationship between strain Sparta and \( M. \text{mycoides} \) subspecies \( \text{mycoides} \).

Additionally, Lemcke (1964) found that Pillai strains G11 and G1/61 were very closely related to \( M. \text{mycoides} \) subspecies \( \text{mycoides} \) while Hudson et al. observed a similar relationship with Y goat strain and 0 goat strain. Their result with Y goat confirmed the original work of Laws (1956), although they found this agent possessed an extra heat-labile antigen.

Tube agglutination test. Relationships found by the agglutination test followed the same pattern. Provost et al. (1964) reported cross reactions between 3 strains of \( M. \text{mycoides} \) subspecies \( \text{mycoides} \) and strains Farcha and Vom. Edward and Leach (Provost et al., 1964) found a relation between \( PG_3 \) antiserum and \( M. \text{mycoides} \) subspecies \( \text{mycoides} \) antigen. Hudson et al. (1967) found no relationship between \( M. \text{mycoides} \) subspecies \( \text{capri} \) (PP goat (Lemcke, 1974)) and \( M. \text{mycoides} \) subspecies \( \text{mycoides} \) although again, as in their complement-fixation work, strains Y and 0 goat cross reacted strongly with \( M. \text{mycoides} \) subspecies \( \text{mycoides} \). Cottew et al. (1969) showed strains BRU, BQT, Smith, Erdel, and N108 were not related to \( M. \text{mycoides} \) subspecies \( \text{mycoides} \), while OSB42, Vom, and other strains, Cov2, Cov3, Razi, and Yam B, were closely related. Apart from partial and one-way reactions with OSB42 and Vom, their Chu and Beveridge strain (PP goat (Lemcke, 1974)) did not cross react significantly with any strain.
Argaman and Razin (1969) showed PG₃ and FP goat to be related to *M. mycoides* subspecies *mycoides*, while El Naari (1967) found PG₃, M08, OSB42, and GPS to be unrelated, although he found a close association between Pillai strains and *M. mycoides* subspecies *mycoides*.

A limiting factor with the technique has been the tendency for certain antigen preparations to autoagglutinate (Edward, 1953; Lemcke, 1964).

**Fluorescent antibody tests.** There is considerably less information on the relation of agents of CCPP to *M. mycoides* subspecies *mycoides* as studied by fluorescent antibody techniques. Tessler and Yedloutschnig (1972) and Tessler (1973a) found the test to be highly specific. In contrast, Perreau (1971) did not. The latter author, as well as Perreau, Gayt, and Monnier (1969) found strains C11 and Vom to be closely related to *M. mycoides* subspecies *mycoides* by the fluorescent antibody test, while strains OSB42, PG₃, and Farcha showed weak cross reactions. Although in the study of the latter strains only antiserum to *M. mycoides* subspecies *mycoides* was employed.

Erns et al. (1972) found PG₃ to be unrelated to *M. mycoides* subspecies *mycoides* by the indirect fluorescent antibody test. Masiga and Stone (1968), using antiserum to *M. mycoides* subspecies *mycoides*, found no relation between this agent and *M. mycoides* subspecies *capri* (their strain of this agent was not named) by an indirect immunofluorescent technique.

**Immunoelectrophoresis.** Stone and Yedloutschnig (1973), using immunoelectrophoresis, observed several similar precipitation bands between USA caprine isolates, Conn. 143, 169, 208, and 212, and *M. mycoides* subspecies *mycoides*. Cross reactions were also
observed between rabbit anti M108 serum and the USA caprine isolates. Growth and metabolic inhibition tests. Al-Aubaidi et al. (1972) divided strains of the species *Mycoides* into 2 groups by the growth and metabolic inhibition tests (Clyde, 1964; Taylor-Robinson, Purcell, Wong, and Chanock, 1966). Their first group, Group 3, comprised PG₃, BQT, Smith, Farcha, and GPA, and this group was not related to the other goat strains or to *M. mycoides* subspecies *mycoides* by either test. The remaining goat strains were indistinguishable from *M. mycoides* subspecies *mycoides*, and the authors placed them in Group 8, characterized by Y goat strain. Group 8 contained almost all the other isolates previously considered to be *M. mycoides* subspecies capri in addition to several other isolates not associated with CCPP, for example the type strain Y goat. Previously Cottew et al. (1969) studied a limited number of strains by growth-inhibition test and found that certain agents, for example, OSB42 and Vom, were related to *M. mycoides* subspecies *mycoides* in contrast to PG₃ (PP goat (Lemoke, 1974)), BQT, and Smith. Edward (1953), Edward and Leach (Provost et al., 1964), Ernö et al. (1972), and Freundt et al. (1973) found that PG₃ did not react with *M. mycoides* subspecies *mycoides*. Lemcke (1974) confirmed that PG₃ and PP goat could be readily distinguished from *M. mycoides* subspecies *mycoides* by these tests. Provost et al. (1964) observed the Vom and Farcha strains to be unrelated to *M. mycoides* subspecies *mycoides*. However, Villemot and Provost (1959) had earlier reported antiserum to *M. mycoides* subspecies *mycoides* strain Maroua partially inhibited strain Farcha. Growth precipitation test. The growth precipitation test has shown cross reactions between PG₃ and *M. mycoides* subspecies *mycoides* (Ernö et al., 1972; Freundt et al., 1973).
The indirect haemagglutination test was used by Ernö et al. (1972) and Freundt et al. (1973), and revealed a close relationship between *M. mycoides* subspecies *mycoides* (PG₁) and PG₃.

Summary of serological relationships between bovine and caprine *M. mycoides*. The anomalous results varying in degree or totally conflicting derive in part from differences in cultural methods and techniques employed. In addition antisera prepared in different hosts and by the use of different injection schedules very likely stimulated different antibodies. Ideally the same antisera should be employed in all tests. Clearly it is not possible to decide on a definite relationship of all these agents to *M. mycoides* subspecies *mycoides*, but overall PG₃, Farcha, GPS, GPA, ERU, EOT, Erdek, Smith, and N108 had a weaker relationship to *M. mycoides* subspecies *mycoides* than the other caprine *M. mycoides* agents. While this division apparently does not yet have a bearing on whether the particular agent causes CCPP it is of more than theoretical interest. The possibility of those caprine strains closely related to *M. mycoides* subspecies *mycoides* giving rise to antibodies in cattle has not been fully investigated but any cross-reactions resulting with *M. mycoides* subspecies *mycoides* would be of significance in the serodiagnosis of CBPP.

Provost, Villemot, Queval, and Valanza (1959) infected cattle by aerosol with a strain of *M. mycoides* subspecies *capri*. The animals were serologically negative prior to inoculation but within 5-10 days they developed agglutinins detected by slide agglutination test with stained *M. mycoides* subspecies *mycoides* antigen. The agglutinins persisted for 1 month. One of the cattle died 14 days post-inoculation with peripharyngeal oedema and oedematous lymph
The organism inoculated was reisolated from the lymph nodes.

Provost (1966) detected complement-fixing antibodies following inoculation of cattle with caprine strain C11 and another similar strain. Yedloutschnig et al. (1971) found strain 212 inoculated into cattle did not give rise to complement-fixing antibodies, although such antibodies were detectable following inoculation of cattle with strain 169. El Nasri (1971) found that experimental cross-pathogenicity and cross-protection tests involving subcutaneous inoculation of M. mycoides subspecies mycoides and caprine strains in cattle and goats were negative.
OTHER RECENT TECHNIQUES USED TO INVESTIGATE
INTER-RELATIONSHIPS OF THE SPECIES MYCOIDES

Mouse Cross-Protection Tests

In vivo comparison of these agents in mice was pioneered by Smith (1965, 1967, 1969) using passive and active mouse cross-protection tests. His CCPP agent did not protect against M. mycoides subspecies mycoides infection while M. mycoides subspecies mycoides protected against infection with his agent of CCPP.

The findings of Arisoy et al. (1969) are perhaps of special interest since they may have particular relevance to the control of CCPP. They studied several agents of CCPP by cross-protection tests in mice. Mice inoculated intravenously with heat-killed vaccines prepared from BQT, ERU, Erdek, Y, Smith, Ban, Go, M108, Cov3, and Razi conferred significant protection to challenge by BQT. When challenged with Y only the homologous vaccine protected. With Cov3 challenge the homologous vaccine and vaccine to Razi and CCPP strain Erdek conferred protection. However in the latter two experiments not all control mice were susceptible and 'whether the use of a more virulent challenge, or greater numbers of mice would have shown that more of the vaccines protected against these strains is not known'. These results indicated that the separation of CCPP agents into groups by Cottew et al. (1969) and Al-Aubaidi et al. (1972) did not have great relevance to their ability to produce cross-protecting antibodies in mice. Moreover, Arisoy et al. (1969) found M. aapalactiae strain A1K2 protected against CCPP strain BQT; this result conflicts with many in vitro tests.
Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis is a further tool which has been used to investigate the relationships of *M. mycoides* subspecies *mycoides* and *M. mycoides* subspecies *capri*. Razin (1968) found the pattern of *M. mycoides* subspecies *mycoides* resembled but was not identical to that of *M. mycoides* subspecies *capri*, PG3. The patterns of strains PG3 and Y were nearly identical, and slight but consistent differences were found between the PG3 and PP goat patterns. However, the patterns of PG3, PP goat, and Y goat were similar. Lemke (1974) confirmed these differences between PP goat, PG3, and *M. mycoides* subspecies *mycoides*. Perreau (1971) found no difference between the patterns of *M. mycoides* subspecies *mycoides*, Vom, and Cl1. Similarly, Perreau et al. (1972) observed comparable fractions in the patterns of 1FX, PG3, and OSB42.

Rodwell et al. (1973) have shown a correlation between certain protein bands (A and B) and the presence of the rho form of the organism in cultures.
MATERIALS AND METHODS

The following specimens were collected sequentially for cultural examinations: lung lesions, bronchial lymph node, mediastinal lymph node, and, where present, pleural fluid, pericardial fluid, and peritoneal fluid as well as any other macroscopically-affected tissue. The most anatomically red port of a lung lesion was always selected for culture and generally small portions of this type of lesion were removed from several points along the 'leading edge' of larger, more established lesions. Immediately all specimens were divided for mycoplasma and bacterial examination. Viable isolation was attempted from lung lesions only.

Technique for Mycoplasma Isolation

Lesions were finely cut, segmented, and suspended in a minimum volume of broth medium. Fetal suspensions and cultures were screened by dark-field microscopy for the presence of mycoplasma before being plated on solid media and maintained in broth.

J. May and Baker Ltd.
Live goats were bled for serum prior to euthanasia by intrajugular injection of euthatol (200 mg/ml pentobarbitone sodium) and exsanguination by severance of the blood vessels of the neck. After removing the skin, the abdominal wall and one side of the chest wall were opened in the mid-line and reflected laterally. Gross pathological lesions were noted and recorded photographically. Detailed pathology and histopathology were carried out with Dr. G. Kaliner, Pathology Department, Veterinary Research Laboratories, Kabete, and the findings are in press (Kaliner and MacOwan, 1976). The following specimens were collected aseptically for cultural examination: lung lesion, bronchial lymph node, mediastinal lymph node, and, where present, pleural fluid, pericardial fluid, and peritoneal fluid as well as any other macroscopically-affected tissues. The most oedematous red part of a lung lesion was always selected for culture and generally small portions of this type of lesion were removed from several points along the 'leading edge' of larger, more established lesions. Immediately all specimens were divided for mycoplasma and bacterial examination. Virus isolation was attempted from lung lesions only.

Techniques for Mycoplasma Isolation
Lesions were finely chopped and resuspended in a minimum volume of broth medium. Lesion suspensions and exudates were examined by dark-field microscopy for the presence of mycoplasma before being plated on solid medium and inoculated into 3 ml volumes of broth.

1 May and Baker Ltd.
The inoculum was distributed in 3 ml volumes of broth to give final dilutions from $10^{-1}$ to $10^{-6}$ of the inoculum. An equal number of uninoculated broths were set up at the same time.

Cultures were incubated for 1 week at $37^\circ C$, being examined daily by dissection microscope for colonies and by dark-field microscope for branching filaments in broth cultures. Routinely, after 1 week, negative broth cultures were plated and the plates incubated for a further week.

**Purification of Mycoplasma Isolates**

Primary isolates were purified by filtration (450 nm Millipore filter*) and serial subinoculation of a single colony onto fresh solid medium. This was repeated a minimum of 3 times and a maximum of 10 times. A culture of an organism treated in this way is referred to subsequently as 'purified'. In addition purified isolates were subcultured onto solid medium containing no bacterial inhibitors for at least 3 passages, to confirm the organism was not a temporary L-phase of bacteria, likely to revert to bacterial form.

**Techniques for Bacterial Isolation**

Bacterial isolation was undertaken by Mr. W.A. Ashford and Dr. C. Nabholz of the Diagnosis Department, Veterinary Research Laboratories, Kabete. Direct smears of all specimens were examined after staining with Grams and Giemsa. The specimens were plated on McConkey agar and 5 per cent horse blood agar and incubated at $37^\circ C$. In addition other inoculated blood agar plates were incubated anaerobically.

**Techniques for Virus Isolation**

Virus isolation was carried out with the cooperation of Dr I. Solberg of the Virology Department, Veterinary Research Laboratories, Kabete. From 3 outbreaks of CCPP the lung lesions of 5 cases were cultured.

*Millipore (U.K.) Ltd., Wembley.*
Fresh kidney tissue was obtained from newborn kids reared in the laboratory breeding herd. The tissue was trypsinised at 37°C with 0.25 per cent trypsin\(^1\) in calcium and magnesium free phosphate buffered saline (PBS), pH 7.5, for 15 minutes with agitation by magnetic stirrer. The cells were collected by centrifugation, washed in PBS, and resuspended at 5 x 10^5 cells/ml in growth medium. The growth medium was Hanks balanced salt solution containing 0.5 per cent bovine lactalbumin hydrolysate, 1.5 per cent of a 4.4 per cent solution of sodium bicarbonate, 1 per cent yeast extract, 0.1 mg/ml of ampicillin, and 10 per cent goat serum. The goat serum was obtained from the laboratory breeding herd maintained at Kabete. Tissue culture tubes with flying coverslips were seeded with 1 ml of cell suspension.

The growth medium was changed every 5 days. When the cells had become confluent 7 - 14 days later the growth medium was removed and the cells washed with PBS prior to inoculation with 0.2 ml of a 10 per cent (v/v) suspension of lung exudate in maintenance medium. The exudate was obtained by expressing fluid from the oedematous edge of acute lung lesions of CCPP. The inoculum was allowed to adsorb for 1 hour at 37°C before the cells were washed with PBS, and 1 ml of maintenance medium added to each tube. An equal number of tubes was not inoculated but otherwise treated in the same way.

The maintenance medium was Earle’s balanced salt solution containing 0.5 per cent bovine lactalbumin hydrolysate, 2 per cent of a 4.4 per cent solution of sodium bicarbonate, 1 per cent yeast extract, and 0.1 mg/ml ampicillin, and 2 per cent goat serum inactivated at 56°C for 30 minutes.

Tissue cultures were studied daily for cytopathic effect before

\(^1\) Difco Laboratories, Detroit, Michigan, U.S.A.
removal of the coverslip which was stained by Giemsa for further microscopy. In addition every 2 days one tube was taken for mycoplasma isolation procedures. The cells in this tube were scraped into the maintenance medium and the suspension studied by dark ground microscopy prior to being plated on VFG (viande-foie goat) solid medium.
MYCOPLASMA STRAINS

Five reference mycoplasma strains were used in addition to 2 strains isolated during this study. The origin of the strains and their donors are shown in Tables 3 and 4.

For further study and classification F30 and F38 strains, isolated during this study, were sent to 3 International Mycoplasma Reference Centres:

Dr. R.H. Leach, Mycoplasma Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW9,(appendix). Professor E.A. Freundt, FAO/WHO International Reference Centre for Animal Mycoplasma, Institute of Medical Microbiology, Bartholin Building, University of Aarhus, D.K. 8000, Aarhus C, Denmark, (appendix).

Dr. G.S. Cottew, CSIRO, Animal Health Research Laboratory, Private Bag No. 1, P.O. Parkville, Victoria 3052, Australia.
Table 3. Reference mycoplasma strains

<table>
<thead>
<tr>
<th>Agent</th>
<th>Disease</th>
<th>Country of origin</th>
<th>Received from</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG₃</td>
<td>CCPP</td>
<td>Turkey</td>
<td>Dr R.H. Leach*</td>
</tr>
<tr>
<td>Smith</td>
<td>CCPP</td>
<td>Turkey</td>
<td>Dr G.R. Smith*</td>
</tr>
<tr>
<td>N108</td>
<td>CCPP</td>
<td>Nigeria</td>
<td>Dr W.N. Masiga*</td>
</tr>
<tr>
<td>Vom</td>
<td>CCPP</td>
<td>Nigeria</td>
<td>Freeze-dried culture collection*</td>
</tr>
<tr>
<td>M. mycoides</td>
<td>CBPP</td>
<td>Australia</td>
<td>Dr W.N. Masiga*</td>
</tr>
</tbody>
</table>

M. mycoides subspecies

mycoides (Gladysdale)

Key:—

* Dr R.H. Leach, Mycoplasma Reference Laboratory, Central Public Health Laboratory, Colindale, London NW9.

Dr G.R. Smith, The Zoological Society of London, Nuffield Institute of Comparative Medicine, Regent's Park, London, NW1 4RY.

Freeze-dried culture collection, Veterinary Research Laboratories, Kabete, Kenya, 1962-1970; received from Dr E.P. Lindley, Federal Department of Veterinary Research, Vom, Northern Nigeria.

Dr W.N. Masiga, East African Veterinary Research Organisation, Muguga, Kenya.
Table 4. Strains isolated in Kenya

<table>
<thead>
<tr>
<th>Agent</th>
<th>Disease</th>
<th>Country of origin</th>
<th>Received from</th>
</tr>
</thead>
<tbody>
<tr>
<td>F30</td>
<td>chronic CCPP</td>
<td>Kenya</td>
<td>Isolated in the course of this study</td>
</tr>
<tr>
<td>F38</td>
<td>acute CCPP</td>
<td>Kenya</td>
<td>Isolated in the course of this study</td>
</tr>
</tbody>
</table>

Experimental animals were housed separately in similar conditions to those of the infected group. The two groups were looked after by different attendants.

Species. All males employed were of the Holstein or cross-bred Holstein type, ranging from 9 to 18 months of age, unless stated otherwise. The sheep were adult, 2 of the ‘Dom’ type and 12 of the ‘Native’ breed. Five to 7 month old Jersey calves were used in the experiment to investigate the pathogenicity of F30 via the intratracheal route while 4 to 8 month old cross-bred Argyshire calves were inoculated subcutaneously with this organism.

All infective inocula were high-passage broth cultures of the Mycoplasma species, while control inocula constituted broth media. All inocula were pasteurised free of bacteria.

Mycoplasma isolated from experimental animals were tested by growth inhibition with growth-inhibiting factors to the organisms inoculated.
PATHOGENICITY EXPERIMENTS

Experimental Animals

Source. All experimental animals were either reared in isolation on the Veterinary Research Farm, Kabete, or brought from farms with herds known to be free of contagious caprine and bovine pleuropneumonia and kept in isolation for at least 2 months prior to experiment. Routinely before inoculation all experimental animals were treated with thiabendazole¹ and Amprol² and their faeces examined for worm eggs and coccidial cocci. Experimental animals were bled prior to inoculation and at intervals thereafter. Experimental groups were balanced as far as possible for breed, sex, and age. Control animals were housed separately in similar conditions to those of the infected group. The two groups were looked after by different attendants.

Species. All goats employed were of the Galla or cross-bred Galla type, ranging from 9 to 18 months of age, unless stated otherwise. The sheep were adult, 6 of the 'Down' type and 18 of the Merino breed. Five to 7 month old Jersey calves were used in the experiment to investigate the pathogenicity of F30 via the intratracheal route while 4 to 8 month old cross-bred Ayrshire calves were inoculated subcutaneously with this organism.

Inocula and Organisms Reisolated from Experimental Animals

All infective inocula were hightitre broth cultures of the mycoplasma studied, while control inocula constituted uninoculated broth medium. All inocula were confirmed free of bacteria.

Mycoplasma isolated from experimental animals were tested by growth inhibition with growth-inhibiting serum to the organism inoculated.

¹ & ² Merck, Sharp, and Dohme Limited, Hoddesdon, Hertfordshire.
**Experimental Designs**

Pathogenicity for Goats by the Intratracheal Endobronchial Route

These experiments were of 2 similar designs:

(1) In each of the first 6 experiments two groups of 12 goats were employed, (Table 5). Within each infected group 8 goats received broth culture followed by a volume of sterile broth via the intratracheal endobronchial route (Abdulla and Lindley, 1967). A further 4 goats were kept in close contact with the inoculated animals. The control group contained the same number of goats, of which 8 were inoculated in this way with sterile broth of the same total volume as the infective inoculum.

(2) In experiment 7 this design was altered by having 6 inoculated animals in the infected group and 6 in-contact goats (Table 5). The control group contained the same number of goats, of which 6 were inoculated in this way with sterile broth of the same total volume as the infective inoculum.

Goats in the infected group of experiment 4 were examined haematologically. A blood sample was taken every 2 days into anticoagulant (disodium salt of ethylenediamine tetra-acetic acid) from each goat.

Pathogenicity for Goats following Intramuscular Inoculation

In each of the first 4 experiments 6 goats in the group to be infected received broth cultures into the right thigh muscles (Table 6). The same number of control goats received a similar volume of sterile broth at this site.

In the fifth experiment two groups of 10 goats were used in this way, while in experiment 4 goats in the infected group were examined haematologically. A blood sample was taken every 2 days into anticoagulant (disodium salt of ethylenediamine tetra-acetic acid) from each goat.
Table 5. Details of endobronchial inoculation experiments in goats

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Agent</th>
<th>Details of culture</th>
<th>Infective inoculum</th>
<th>Duration of expt (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3rd pass</td>
<td>Vol of culture</td>
<td>Vol of uninoc.</td>
</tr>
<tr>
<td>1</td>
<td>M.G. *</td>
<td>10 ml</td>
<td>7.5 ml</td>
<td>2.5 x 10^{10}</td>
</tr>
<tr>
<td>2</td>
<td>N108</td>
<td>10 ml</td>
<td>7.5 ml</td>
<td>8.8 x 10^{9}</td>
</tr>
<tr>
<td>3</td>
<td>PG3</td>
<td>10 ml</td>
<td>7.5 ml</td>
<td>7.5 x 10^{8}</td>
</tr>
<tr>
<td>4</td>
<td>Vom</td>
<td>10 ml</td>
<td>7.5 ml</td>
<td>6 x 10^{10}</td>
</tr>
<tr>
<td>5</td>
<td>F30</td>
<td>5 ml</td>
<td>10 ml</td>
<td>1 x 10^{9}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>purified 10X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F38</td>
<td>20 ml</td>
<td>10 ml</td>
<td>1 x 10^{10}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>purified 6X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>pooled</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F38</td>
<td>10 ml</td>
<td>10 ml</td>
<td>2 x 10^{8}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>purified 6X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*M.G.: M. mycoides subspecies mycoides (Gladysdale)

cfu: colony forming units
Table 6. Details of intramuscular inoculation experiments in goats

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Agent</th>
<th>Details of culture</th>
<th>Infective inoculum</th>
<th>Duration of expt (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M.G.*</td>
<td>3rd pass</td>
<td>3 ml</td>
<td>$1.5 \times 10^9$</td>
</tr>
<tr>
<td>2</td>
<td>N108</td>
<td>3rd pass</td>
<td>2 ml</td>
<td>$1.8 \times 10^9$</td>
</tr>
<tr>
<td>3</td>
<td>PG3</td>
<td>3rd pass</td>
<td>2 ml</td>
<td>$7.5 \times 10^8$</td>
</tr>
<tr>
<td>4</td>
<td>Vom</td>
<td>4th pass</td>
<td>3 ml</td>
<td>$1.8 \times 10^{10}$</td>
</tr>
<tr>
<td>5</td>
<td>F30</td>
<td>18th pass</td>
<td>1 ml</td>
<td>$1.5 \times 10^9$</td>
</tr>
</tbody>
</table>

Purified 10X

*M.G.: M. mycoides subspecies mycoides (Gladysdale)*

Pathogenicity for sheep. In the group to be infected 5 adult
sheep were inoculated with 1 ml of F30 1st pass, purified, broth
culture containing $6 \times 10^8$ colony forming units (c.f.u.) and 9 ml
of sterile broth via the intratracheal route, and 4 others were kept
in close contact. The control group contained the same number of
animals, 3 of which were inoculated in this way with sterile broth.
Survivors were slaughtered 60 days post inoculation (p.i.), 43 days
after the death of the last clinically-affected sheep.

Pathogenicity for cattle. Two calves each received 100 ml of low
pass, purified, F30 culture containing $1.5 \times 10^9$ c.f.u intratracheally
while 2 control calves received the same volume of sterile broth via
this route. The inoculated calves were slaughtered 42 days later.

Post calves were each inoculated with 1 ml of low pass, purified
F30 broth culture containing $3.5 \times 10^8$ c.f.u/ml, subcutaneously behind
the right shoulder. A control group of 4 calves were treated in the
Further Experiments with the F30 Strain

Contact transmission. Eight goats were maintained in close contact with 1 inoculated donor goat. The in-contact goats were of the Galla type, 7 ranging in age from 5 to 8 months, while the eighth was a 1-month suckling kid. Following the death of the donor animal another was introduced. The first 3 donor goats were inoculated via the intratracheal route with 1 ml of F30 culture and 5 ml of sterile broth, the next 3 received the same inoculum via the intrapulmonary route, and the final 3 were inoculated via the intratracheal endobronchial route with 5 ml of F30 culture followed by 5 ml of sterile broth. The contact animals were exposed to an inoculated and affected donor goat for 66 days, and they were killed 42 days after the death of the last donor goat.

Pathogenicity for sheep. In the group to be infected 8 adult sheep were inoculated with 1 ml of F30 low pass, purified, broth culture containing $6 \times 10^8$ colony forming units (c.f.u) and 9 ml of sterile broth via the intratracheal route, and 4 others were kept in close contact. The control group contained the same number of animals, 8 of which were inoculated in this way with sterile broth. Survivors were slaughtered 60 days post inoculation (p.i.), 43 days after the death of the last clinically-affected sheep.

Pathogenicity for cattle. Two calves each received 100 ml of low pass, purified, F30 culture containing $1.5 \times 10^9$ c.f.u intratracheally while 2 control calves received the same volume of sterile broth by this route. The inoculated calves were slaughtered 69 days p.i..

Four calves were each inoculated with 3 ml of low pass, purified F30 broth culture containing $3.2 \times 10^9$ c.f.u/ml subcutaneously behind the right shoulder. A control group of 4 calves were treated in the
same way with sterile broth. Two inoculated calves were slaughtered 28 days p.i., while the remaining 2 were killed 103 days p.i..

In both experiments calves were bled prior to inoculation and weekly thereafter, their serum being stored at -20°C for complement fixation tests.

Further Experiments with F38 Type Isolate G69

The F38 type isolate, G69, was derived from a more recent acute outbreak of contagious caprine pleuropneumonia and it was inhibited by growth inhibiting serum prepared in rabbits to strain F38.

Contact transmission following intravenous inoculation. The agent had been purified 3 times and was at the 10th pass in artificial medium at the time of inoculation. One goat received 0.5 ml of chloroform intravenously (Longley, 1951), followed 2 hours later by 3 ml of broth culture containing $7 \times 10^7$ c.f.u. Fifteen days p.i. 7 healthy goats were placed in close contact with the inoculated goat. Also on day 15, three adult goats were inoculated as above and added to the experiment. All goats were bled for serum prior to being included in the experiment and weekly thereafter.

Contact challenge experiment with strains F38 and G69. After the first 7 in-contact animals had become affected in the G69 contact transmission experiment (above) the 12 goats used in the first F38 intratracheal endobronchial experiment were introduced in contact.

At the same time 5 other healthy goats were placed in contact. To ensure continuity of in-contact transmission two other groups of 3 goats each were added to the experiment 28 days and 42 days later respectively.
A G69 intratracheal endobronchial and contact experiment with stringent controls. The infective inoculum was a broth culture of isolate G69 from an acute contact case in the preceding experiment. This inoculum was purified 3 times and shown to be the same as the original isolate G69 and strain F38 by growth and metabolic inhibition test using growth inhibiting serum to strain F38 prepared in rabbits. The broth culture inoculated was at the 16th pass in artificial medium and each of 10 goats in the infected group received 20 ml containing $2.6 \times 10^9$ cfu intratracheal endobronchially (Abdulla and Lindley, 1967).

The infected group contained 10 inoculated goats and 10 in contacts. The control group contained the same number of goats of which 10 were inoculated in this way with sterile broth of the same volume as the infective inoculum.

Division of goats into infected and control groups was effected by random selection of 4 groups of 10 from a pool of 40 (Table 0, Rohif and Sokal, 1969) so that each goat in the infected group was paired with a similarly treated control animal.

When a goat in the infected group was killed or died its 'control pair' was killed on the same day and similar samples from both animals were cultured for mycoplasma and bacteria.

All goats were culturally examined for P13 virus by Dr. I. Solberg of the Virology Department, Veterinary Research Laboratories, Kabete. Before inoculation nasal swabs from all goats were soaked in 2 ml of phosphate buffered saline, pH 7.5 which contained 500 i.u. penicillin, 500 ug streptomycin, 200 ug neomycin and 200 ug mycostatin per ml. During the experiment nasal swabs were also taken from febrile goats in the infected group on the second day of fever.
At post-mortem examination retropharyngeal lymph nodes from all goats together with the lung lesions of goats in the infected group were sampled for virus isolation. From each site 1 g of affected tissue was ground in sterile sand with 2 ml of the antibiotic solution similar to that used for nasal swabs.

All specimens were stored from 1 to 21 days at -20°C before inoculation into 2nd generation bovine embryo kidney monolayers in roller tubes. Cultures were incubated at 37°C and observed daily for cytopathic effect. After 10 days incubation haemadsorption (HAD) tests were carried out at 4°C for 30 minutes using 0.4% washed guinea pig red blood cells.

To identify cytopathic haemadsorbing agents as PI₃ virus the haemadsorption inhibition test was used with a hyperimmune serum prepared to a strain of bovine PI₃ virus. The hyperimmune serum was supplied by Dr. J. Kaminjolo, Faculty of Veterinary Medicine, University of Nairobi.

For haematological study samples were taken into EDTA from all goats 3 times at a 2 day interval, prior to the experiment.

An experiment to enable further serological study of G69 contact cases. An additional 8 healthy goats were put in contact with the infected group of the previous experiment 7 days before the last contact case was killed. As controls another 8 goats were maintained in a separate building. All goats were bled for serum prior to the experiment, and also the 8 in contact goats were bled every second day after the onset of fever.
SEROLOGICAL METHODS

Preparation of Complement Fixation and
Agar Gel Diffusion Test Reagents

Antigens
Organisms other than F38 type isolates were cultured in 2-litre volumes of Newing's tryptose broth containing 10 per cent (v/v) rabbit serum which had been heated at 56°C for 0.5 hours. It was necessary to culture isolates of the F38 type in VFG50 broth. When the titre exceeded $10^9$ cfu/ml the culture was centrifuged at 40,000 g for 1 hour at 0-5°C. The deposit was resuspended and washed 3 times in physiological saline prior to final suspension in 20 ml of physiological saline and storage in 0.5 -1 ml volumes at -20°C. Sterile broth treated as above constituted the sediment antigen while the second control antigen was freeze-dried broth reconstituted at 200 mg/ml. Tissue antigens were prepared by fine mincing and suspension in a minimum volume of physiological saline. Contagious bovine pleuropneumonia lung lesion was supplied by Dr J. Soudamore of the CBPP Department, Veterinary Research Laboratories, Kabete.

Antisera
Hyperimmune antisera prepared in rabbits for the complement fixation and agar gel diffusion tests were prepared with the culture antigens described above by either a modification of the method of Krauss and Wandera (1970), in which complete Freund's adjuvant replaced incomplete Freund's adjuvant or by the method of Lemcke (1965), using 2 injections of Freund's complete adjuvant and antigen.

For the preparation of growth-inhibiting rabbit antisera to

---

1 M.S.E. autorefrigerated superspeed centrifuge patent 27599,
10 x 100 ml angle head rotor.
P30, *M. mycoides* subspecies *mycoides* (Gladysdale), PG₃, and N108, broth cultures containing over $10^9$ cfu/ml were also used as antigen. The first 3 injections were as described in the first of the above methods, being followed by weekly intravenous injections of 1 ml of broth culture until growth-inhibiting sera were obtained. With Smith, Vom, and P38 strains hyperimmune serum prepared for agar gel diffusion and complement fixation tests possessed growth-inhibiting activity. The sera of rabbits to be immunized were confirmed negative by the tests for which they were prepared. All rabbits being immunized were bled at weekly intervals and their sera tested for antibody. Two rabbits from the closed laboratory breeding herd were immunized with each strain. After immunization the rabbits were exsanguinated and the sera tested by agar gel diffusion test, complement fixation test, or growth inhibition test where appropriate. From each pair the serum giving the strongest reaction with the homologous organism was selected for comparative serology.

Other antisera included bovine sera from field cases of CBPP with a known high complement fixation titre to *M. mycoides* subspecies *mycoides*, supplied by Dr J. Soudamore of the CBPP Department, Veterinary Research Laboratories, Kabete.

For all serological tests rabbit and bovine sera were inactivated at $56^\circ$C for 0.5 hours, while goat sera were inactivated for the same period at $60^\circ$C.

**Serological Tests**

The results recorded for the various serological procedures represent the same finding from 2 or more consecutive tests.
In other words a particular serological test was repeated until the result was the same in two or more tests performed serially.

**Agar Gel Diffusion Test**

Molten 2 per cent (w/v) agarose was mixed with an equal volume of double-strength barbitone buffer, pH 8.2 (Kohn, 1968), and 3 ml volumes pipetted onto agar-coated standard microscope slides. Well patterns with 1 central and 6 peripheral wells 7 mm apart and 4 mm in diameter were prepared. All antigens were frozen and thawed 6 times in an acetone and dry ice bath. Precipitation lines were read after 48 hours at room temperature in a moist chamber. For record purposes agar gel slides were dried and stained. The agar slides were washed for 48 hours in 0.85 per cent (w/v) sodium chloride, followed by 48 hours in distilled water, and then covered with filter paper and dried for 72 - 96 hours at room temperature. Dried slides were immersed for 15 minutes in 1 part by weight Amido Schwarz 10B in 1000 parts 5 per cent (v/v) glacial acetic acid, and decolourization accomplished by several washings in a mixture of 20 parts by volume glacial acetic acid, 50 parts methanol, and 50 parts water.

Sera from experimental goats were concentrated with the aid of Dr. C. Staak, Department of Parasitology, Veterinary Research Laboratories, Kabete. Sera in 8/32" Visking dialysis tubing *1 were concentrated twofold with Acquacide *2 for 10 minutes per ml of serum. The concentrated serum was then dialysed against veronal buffer pH 7.3 (Williams and Chase, 1968) overnight at 4°C using a magnetic stirrer. Each ml of serum was dialysed against 250 ml of veronal buffer.

---

*1 The Scientific Instrument Centre Ltd., 1 Leeke Street, London WC1.
*2 Calbiochem AG, Loewengraben 14, 6000 Lucerne 5, Switzerland.
Complement Fixation Test

Microtitre plate tests were performed by the method of Grist, Ross, Bell, and Stott (1966) using 0.025 ml volumes, 2 volumes of complement containing 6 minimum haemolytic doses, and a 1.5 per cent (v/v) final concentration of red blood cells.

When the complement fixation test was used to examine goat sera only 3 minimum haemolytic doses of complement were employed.

Tube Agglutination Test

The technique was as described by Cottew et al. (1969).

Agglutination tests were carried out in tubes using equal volumes (0.25 ml) of serum dilution and antigen, opacity matching Brown tube 2. The reagents were mixed by shaking and the tubes were incubated at 37°C overnight. After readings were made, the tubes were returned to the incubator and were read again 24 hours later.

Growth Precipitation Test

This test was carried out as described by Krogsgaard-Jensen (1972) except that Newing's tryptose agar containing 30 per cent inactivated pig serum was used as the solid medium. For the growth of reference mycoplasma and F30 Newing's tryptose broth with reduced glucose and 20 per cent inactivated pig serum was used, while F38 was grown in VFG50 broth.

Plates were poured to give solid medium 3 to 3.5 mm deep and wells 6 mm in diameter were cut with a copper tube and then filled with mycoplasma broth culture. Six mm diameter filter paper discs were placed on the agar surface 1 cm from the rim of the well and the plates were incubated at 37°C in a humid atmosphere for up to 8 days. A positive reaction gave 1 or more precipitation lines in the agar between the well and the filter paper disc.
Growth Inhibition Test

The method employed was a modification of the procedure described by Clyde (1964). A solid medium plate was placed open in a 37°C incubator for 1 hour to dehydrate the medium surface after which the plate was inoculated with a dilution of log phase broth culture selected to give numerous but not confluent colonies. Wells 6 mm in diameter were cut in the agar using a sterile metal tube and filled with undiluted hyperimmune serum prepared in rabbits. The plate was reincubated until colonies became visible and examined for zones of inhibition about the wells. Wells filled with normal rabbit serum served as controls.

Metabolic Inhibition Test.

The microtitre plate metabolic inhibition test was used (Taylor-Robinson et al., 1966). For the F38 strain VFG50 broth with 1% glucose was employed while F30 and the reference strains were tested in Newing's tryptose broth with 0.5% glucose. A final concentration of 6% fresh guinea pig serum was included in all wells, and tests were incubated at 37°C.
Newing's Tryptose Medium


A slight modification of this medium was used to culture all reference mycoplasma strains. For culture of the caprine reference strains and F30 the concentration of glucose in broth was reduced by half.

The base consisted of Bacto tryptose Difco 2.0 per cent (w/v), sodium chloride 0.5 per cent (w/v), anhydrous sodium hydrogen phosphate 0.25 per cent (w/v), dissolved in distilled water to 100 per cent by steaming for 20 minutes. The pH was adjusted to 8 before Seitz filtration and then readjusted to 7.6. Finally 10 ml 0.1 per cent phenol red was added per litre (Cruickshank, 1965).

For broth the base was dispensed in 153 ml volumes, while for solid medium agar\(^1\) was added to a final concentration of 1.5 per cent and the mixture dispensed in volumes of 392 ml. The dispensed volumes were sterilised by autoclave at 15 lb/square inch for 15 minutes and stored at room temperature.

All additive stock solutions (Table 7) except penicillin and serum were dissolved in distilled water with heating where necessary, dispensed in 20 ml volumes, and sterilized by autoclave at 10 lb/square inch for 15 minutes before storage at room temperature.

Penicillin was dissolved in distilled water and kept at 4°C.

Serum was clarified by centrifugation and Seitz-filtered before storage at -20°C. Serum sterility was confirmed by incubating inoculated liquid and solid mycoplasma medium and 5

\(^1\)Oxoid Ltd., London.
Table 7. Additives for Newing's tryptose media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Additive</th>
<th>Stock concentration</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid</td>
<td>Glucose</td>
<td>$50%$ $(w/v)$</td>
<td>$0.5%$ $(w/v)$</td>
</tr>
<tr>
<td></td>
<td>Bacto-yeast</td>
<td>$10%$ $(w/v)$</td>
<td>$0.1%$ $(w/v)$</td>
</tr>
<tr>
<td></td>
<td>extract (Difco)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thallium acetate</td>
<td>$5%$ $(w/v)$</td>
<td>$0.5%$ $(w/v)$</td>
</tr>
<tr>
<td></td>
<td>Penicillin</td>
<td>$5 \times 10^6$ iu/100 ml</td>
<td>100 iu/ml</td>
</tr>
<tr>
<td></td>
<td>Glycerol</td>
<td>neat</td>
<td>$0.5%$ $(v/v)$</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>neat</td>
<td>$20%$ $(w/v)$</td>
</tr>
<tr>
<td>Solid</td>
<td>Glucose</td>
<td>$50%$ $(w/v)$</td>
<td>$0.5%$ $(w/v)$</td>
</tr>
<tr>
<td></td>
<td>Bacto-yeast</td>
<td>$10%$ $(w/v)$</td>
<td>$0.1%$ $(w/v)$</td>
</tr>
<tr>
<td></td>
<td>extract (Difco)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Penicillin</td>
<td>$5 \times 10^6$ iu/100 ml</td>
<td>100,000 iu/100 ml</td>
</tr>
<tr>
<td></td>
<td>Thallium acetate</td>
<td>$5%$ $(w/v)$</td>
<td>$0.01%$ $(w/v)$</td>
</tr>
<tr>
<td></td>
<td>Glycerol</td>
<td>neat</td>
<td>$0.5%$ $(v/v)$</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>neat</td>
<td>$30%$ $(v/v)$</td>
</tr>
</tbody>
</table>
per cent horse blood agar plates. The cultures were incubated for 1 week before the serum was considered sterile. Serum was always stored at -20°C but other additives which were in current use were kept at 4°C. Immediately before addition to medium serum was always inactivated at 56°C for 0.5 hours.

Sera from the following species were employed in Newing's tryptose medium: pig, horse, goat, ox, and rabbit. Routinely pig serum was employed.

To prepare solid medium the agar base was melted and allowed to cool to 56°C prior to mixing in preheated additives, and pouring the plates. All prepared media were kept at 4°C.

Viande-foie Goat Medium (VFG).

The medium was a modification of that described by Al-Aubaidi and Fabricant (1968) and derived from Turner et al (1935).

The base consisted of 100 gm of goat muscle and 100 gm of goat liver tissue, together with 120 gm of fresh pig stomach. The tissues were cut into small pieces and finely minced. To this mixture 1 litre of distilled water and 10 ml of concentrated hydrochloric acid were added while stirring. The suspension was incubated at 50°C for 24 hours, then heated to 80°C, and filtered through a clarifying filter paper. The filtrate was again heated to 80°C and held at 4°C overnight. The cold solution was passed through filter paper, the pH adjusted to 7.6 with 10% (w/v) sodium hydroxide, and warmed to 80°C. One per cent of buffer salt mixture (379.0 gm anhydrous disodium phosphate and 90.8 gm potassium dihydrogen phosphate) was added. The buffered mixture was held at 4°C overnight, filtered through filter paper, and the pH readjusted to 7.6. Thallium acetate and phenol red (as for Newing's tryptose medium were added (Table 7) and the base Seitz filtered before storage at 4°C.
The whole medium comprised VFG base to which glucose, yeast extract, glycerol and penicillin were added in the same concentrations as for Newing's tryptose medium (Table 7) as well as goat serum. For broth 50% inactivated sterile goat serum was added, whereas 30% inactivated sterile goat serum and a final concentration of 1.5% agar Noble were included for solid medium. The broth was referred to as VFG50 and the solid medium as VFG agar.

Concurrently each organism was inoculated into broth without sugar and broth with serum, and an equal number of inoculated and uninoculated broths were set up. All inoculated broths received 1 part by volume of culture to 9 parts of broth before inoculation and daily thereafter, the pH of each broth was measured with a pH meter. In addition inoculated broths were plated daily on solid medium. After 7 days the experiment was terminated and all broth checked for the absence of contaminating bacteria.

Hemolytic

In the first technique organisms were grown on solid medium without thallium acetate, containing 7 per cent (v/v) defibrinated blood, either horse, goat, sheep, guinea pig, or chicken (Coffey et al., 1969). In the second method, well-separated, young colonies were thinly overlaid with 3 per cent (v/v) PHL agar containing 5 per cent (v/v) of the same type of blood before reinoculation.

Preserve Preparation

All organisms were tested for production of proline (Van, Well, and Marits, 1966) on Agar's modified agar containing 2% blood cells and no thallium acetate. Inocula were prepared by
Fermentation Tests

Log-phase cultures of the strains tested were inoculated into broths containing 1 per cent (w/v) of one of the following substrates:

- glucose
- laevulose
- mannose
- maltose
- trehalose
- starch
- dextrin
- glycogen
- sorbitol
- arabinose
- xylose
- rhamnose
- adonitol
- galactose
- mannitol
- dulcitol
- sucrose
- raffinose
- inulin
- salicin

Concurrently each organism was inoculated into broth without sugar and broth without serum, and an equal number of inoculated and uninoculated broths were set up. All inoculated broths received 1 part by volume of culture to 9 parts of broth. Before inoculation and daily thereafter the pH of each broth was measured with a pH meter. In addition inoculated broths were plated daily on solid medium. After 7 days the experiment was terminated and all broths checked for the absence of contaminating bacteria.

Haemolysis

In the first technique organisms were grown on solid medium without thallium acetate, containing 5 per cent (v/v) defibrinated blood, either horse, goat, sheep, guinea pig, or chicken (Cottew et al., 1969). In the second method, well-separated, young colonies were thinly overlaid with 1 per cent (w/v) PPLO agar containing 5 per cent (v/v) of the same defibrinated bloods before re-incubation.

Peroxide Production

All organisms were tested for production of peroxide (Cole, Ward, and Martin, 1968) on Newing’s tryptose agar containing horse red blood cells and no thallium acetate. Inocula were prepared by
scraping young confluent colonies from solid medium. To test F18, colonies were overlaid with 1 per cent (w/v) PPLO agar containing red blood cells and benzidine dihydrochloride.

**Methylene Blue Reduction**

The organisms were tested in Newing's tryptose broth as described by Edward (1950). Broth cultures to which 0.4 ml of a 1/10,000 solution of methylene blue had been added were incubated at 37°C and examined daily.

**Liquefaction of Inspissated Medium**

The technique detailed by Jonas and Barber (1969) was followed using Newing's tryptose broth including either 40 per cent (v/v) goat serum, 30 per cent (v/v) bovine serum, or 30 per cent (v/v) pig serum, instead of the usual serum supplement.

**Growth in Medium without Serum**

Mycoplasma broth and agar containing no serum were inoculated with log-phase cultures of F30 or F38 and incubated for 1 week. The broth cultures were subcultured several times and reincubated for the same period. The number of viable organisms was assessed by plate count at 2-day intervals.

**Sensitivity to Digitonin.**

This test was carried out as described by Ernö and Stipkovits (1973) using Newing's tryptose and VFG media.

**Sensitivity to Antibiotics**

Solid medium was flooded with a known dilution of a log-phase broth culture, allowed to dry, and an Oxoid Multodisk placed on the seeded surface prior to incubation. Inhibition of colony formation around the antibiotic impregnated disk was interpreted as sensitivity

---

1 Difco
2 Oxoid Limited, London.
to that antibiotic. The following antibiotics were tested: alpha tetracycline, 10 mcg; chloramphenicol, 10 mcg; furazolidone (neftin) 15 mcg; neomycin, 10 mcg; penicillin, 1.5 units; ampicillin, 2 mcg; streptomycin, 10 mcg; a sulphonamide, 50 mcg.

In addition F30, F38, FG3, N108, and M. mycoides subspecies mycoides (Gladysdale) were grown in broth containing 100 mg/ml of ampicillin. The cultures were set up in parallel with an equal number without ampicillin. Each strain was passaged 8 times and the effect of ampicillin assessed by plate counting the cultures.

Viable Count or Plate Count Technique

The method of Miles and Misra (1938) was modified: 4 solid medium plates were dried for 1 hour at 37°C and each dilution of culture counted was dispensed in 6, 0.025 ml drops on 1 plate. Disposable microtitre complement-fixation test droppers \(^1\) were used to deliver the drops.

Viability Tests

At 4°C. High-titre cultures were placed at 4°C and viability assessed by viable counts at weekly intervals. Each agent was tested twice.

At -20°C. Routinely, high-titre broth cultures of all mycoplasma strains were stored at -20°C.

Freeze-dried cultures. High-titre broth cultures in 0.25 ml volumes were dried using an Edwards freeze drier with phosphorous pentoxide as dessicant. At intervals cultures were reconstituted and cultured on solid medium to assess viability.

Culture in Embryonated Eggs

0.1 ml of each of 4 serial tenfold dilutions of a log-phase broth culture were inoculated via the yolk sac. Each dilution was

\(^1\) Flow Laboratories, Irvine, Scotland.
inoculated into two 7-day embryonated hen's eggs. The highest dilution contained less than 1 colony forming unit. Four similar eggs were inoculated with sterile broth and 4 others maintained as control eggs. The embryonated egg killed by the highest dilution was used as inoculum for the next pass. Fifty passes of F30 were conducted using aspirated infected yolk sac fluid as the inoculum. Inocula and harvests were examined for the presence of contaminating bacteria. The harvest from the fiftieth pass was grown in broth and tested in the growth-inhibition test against homologous serum before being intramuscularly inoculated into 3 goats.

**Comparative Pathogenicity Tests in Embryonated Eggs**

In each experiment 70 embryonated eggs, 7–9 days old, were divided into groups of 10. Five groups were inoculated by the yolk sac route with 0.1 ml of a tenfold dilution of a log-phase broth culture of the mycoplasma under test, so that the highest dilution contained less than 1 organism. Of the remaining 2 groups, 1 received 0.1 ml of sterile broth medium via the same route, while the other was not inoculated. Yolk from eggs which died during the experiment were plated on 5 per cent horse-blood agar and mycoplasma solid medium for 1 week at 37°C.
MORPHOLOGICAL EXAMINATION

Microscopy
Colonies were examined after 48, 96, and 192 hours, while broth cultures were examined after 24, 48, 96, and 192 hours.

Light microscopy. Colonies were studied with a dissecting microscope. Sections of F30 colonies were examined with a standard microscope, the sections being stained with toluidine blue following preparation as for electron microscopy. Wet preparations from broth cultures were examined by dark-field and phase-contrast microscope. Wet preparations were photographed with a microscope in phase contrast using a microflash.¹

Electron microscopy of F30. Part of this work was carried out with the advice and assistance of Professor H. Krauss, Pathology Department, Veterinary Faculty, University of Nairobi. Single colonies were cut out from suitable plates and fixed with 5 per cent glutaraldehyde in 0.2M cacodylate buffer for 1 hour at 4°C, or fixed with osmium tetroxide as described by Kellenberger, Ryter, and Sechaud (1958). Colonies fixed with glutaraldehyde were postfixed with 1 per cent osmium tetroxide in 0.2M cacodylate buffer for 1–2 hours, after washing 3 times for 5 minutes each in the same buffer.

Fifty ml volumes of high titre broth culture were centrifuged at 10,000 g at 4°C for 1 hour. Half the cultures were fixed at 4°C for 30 minutes before centrifugation by adding, dropwise, 25 per cent glutaraldehyde under continuous stirring to give a final

¹ Leitz Ortholux microscope, Leitz microflash and Kodak 125ASA film.
Concentration of 1 per cent. Pellets from the other cultures were fixed in the same way as the colonies with 5 per cent glutaraldehyde. All pellets were postfixed with 1 per cent osmium tetroxide in 0.2M cacodylate buffer for 1 - 2 hours.

After the fixation procedure, specimens were rinsed in 0.1M sodium acetate, treated with 0.5 per cent uranyl acetate for 20 minutes and rinsed again with 0.1M sodium acetate. The specimens were dehydrated with acetone and embedded in Durcupan\(^1\) in such a way that colonies could be cut in cross section. Phase sections of 1 \(\mu\)m thickness were cut with glass knives on an Ultramicrotome\(^2\), stained with toluidine blue, and observed under a light microscope. Thin sections of 50 to 90 \(\mu\)m mounted on formvar-coated copper grids were stained with lead citrate and examined with a Zeiss EM 9A electron microscope.

Sections were prepared from all parts of each colony.

**Negative staining.** An area of agar showing confluent 48-hour colonies of F30 was flooded with 5 per cent glutaraldehyde in 0.2M cacodylate buffer for 1 hour at 4\(^\circ\)C. Material from fixed colonies was transferred to a drop of 1 per cent phosphotungstic acid in 0.4 per cent sucrose for 1 minute, and finally mounted on carbon-coated grids by touching the drop with the grid.

---

1 Durcupan: Fluka, Basel, Switzerland.

2 OMU2 Reichart Ultramicrotome.
RESULTS

Most of the affected cases occurred early in the fall in the Veterinary Research Laboratories facilities, in terminal condition. In addition, none of the affected cases were dispatched. The number of cases and specimens submitted in no way reflected the incidence of the disease, because the disease was widely recognized in the field and diagnosis was not a problem. The condition was diagnosed at altitudes varying from sea level to 7,000 ft., and no cases were observed in species other than goats. Nearly all cases were received during the latter, colder, part of the year.

Symptoms

Nasal discharge was not a constant and only in isolated cases was it evident. At clinical examination a cough was not often evident, but in those cases kept under observation an indistinct harsh cough usually became apparent. In one field outbreak, acute cases had rectal temperatures up to 40.3 = 41.0°C, while in other cases where the course was more prolonged rectal temperatures remained within the normal range. In acute fatal cases body condition was not markedly affected but cases with a prolonged course became emaciated, debilitated, and dyspneic, often grinding their teeth for long periods.
DISTRIBUTION AND SEASONAL PREVALENCE

Most of the affected goats examined were sent to the Veterinary Research Laboratories, Kabete, in terminal condition. In addition organs of affected goats were despatched. The number of cases and specimens submitted in no way reflected the incidence of CCPP, because the disease was widely recognized in the field and diagnosis was not a problem. The condition was diagnosed at altitudes varying from sea level to 7,000 ft., and no cases were observed in species other than goats. Nearly all cases were received during the wetter, colder, part of the year.

SYMPTOMS

Nasal discharge was not always present and only in isolated cases was it copious. At clinical examination a cough was not often evident, but in those cases kept under observation an intermittent harsh cough usually became apparent. In one field outbreak, acute cases had rectal temperatures up to 40.5 - 42°C while in other cases where the course was more prolonged rectal temperatures remained within the normal range. In acute fatal cases bodily condition was not markedly affected but cases with a prolonged course became emaciated, debilitated, and dyspnoeic, often grinding their teeth for long periods.
Incubation Period, Course, Morbidity and Mortality

It was difficult to obtain accurate information on the incubation period, course, morbidity, or mortality, because many farmers either sold and/or vaccinated their goats as soon as the disease was recognised.

On several occasions affected goats were purchased and kept under observation at the Veterinary Research Laboratories, Kabete. On arrival the animals were febrile and the majority remained so until death within one week. A few partially recovered, their rectal temperature falling to normal within a fortnight. These animals were usually killed for the preparation of autogenous vaccine before they died or became chronic cases. Two cases were kept under observation for a long period; one died after a month while the other was slaughtered at 3 months. Such results do not reflect the field situation because the goats were subject to the stress of transport and thereafter to husbandry very conducive to convalescence.

Immunity

It was impossible to assess the immune status of indigenous goats due to the problem of inducing experimental disease. At present an experimental autogenous vaccine is prepared from formalinised lung lesions of field cases but the efficacy of this preparation has not been established. In certain areas, particularly the Northern Province, goat herdsmen insert pieces of fresh lung lesion subcutaneously about the head.

Atypical Syndromes of CCPP

No outbreaks or individual cases of disease similar to oedema disease or atypical syndromes of CCPP were reported to or observed
at the Veterinary Research Laboratories, Kabete, during 1970 - 1975.

Macroscopic Lesions

The appearance of the prominent hepatized lesions showed wide colour variations from a fairly even light chocolate brown (Plates 1 and 2) to a striking mosaic with shades of brown, red, blue, yellow, and green (Plates 4 and 5). The texture of the lesions varied from soft and 'liver-like' (Plates 1 and 2) to a very dense and friable consistency (Plates 3 - 6). The centres of large lesions sometimes showed a clear 'water mark' denoting a very necrotic and friable zone (Plates 1 and 2). In some cases the edge of the affected tissue was soft, oedematous, and red, merging with the surrounding unaffected lung (Plates 1 and 2) while in others there was a sharply defined elevated border of harder hepatized tissue (Plate 4). The lesion shown in Plate 5 did not fit either category; it gradually rose above the normal lung to reach a peak in the centre. The gross enlargement of the affected lung tissue was very obvious in Plate 6; one half of the lung was totally affected.

On the cut surface even the advanced friable, yellow parts of hepatized lesions retained some resemblance to normal lung in that the larger air passages remained patent (Plate 7). This figure also shows the merging of oedematous, red areas into older necrotic parts of the lesion. In many cases, besides one or more large lesions smaller oedematous, red, hepatized lesions occurred as discrete foci in other parts of the lung (Plates 1, 2 and 5). Fibrin adhesions frequently involved not only the visceral pleura over lesions and the opposite parietal pleura but also the pericardium
(Plates 3, 4, and 6). In rare cases no fibrin was observed (plates 1 and 2). Clear, yellow pleural fluid was very variable in volume, from little or none up to 0.5 litre. The mediastinal lymph nodes and often the bronchial lymph node were consistently enlarged, oedematous, and sometimes haemorrhagic. In only one case was sero-fibrinous epicarditis and pericarditis observed (Plate 8).

In no case was the nature of the lesion similar to that of contagious bovine pleuropneumonia. Enlargement of interlobular septae was generally not observed macroscopically. In rare cases small areas of interlobular septal enlargement were seen in the oedematous red edge of larger hepatized masses. Neither splenomegaly, changes in cardiac muscle, nor arthritis were observed.

Chronic cases were observed from two herds known to have suffered the acute disease 1 - 3 months previously. Much of the affected lung was replaced by fibrous tissue which enclosed areas of pale, very friable, necrotic tissue varying in size. Frequently the whole of one half of the lung was covered with fibrous tissue joining lobes and attaching the lung to the costal pleura, diaphragmatic pleura, pericardium, and mediastinum (Plate 9).
Field Cases of Contagious Caprine Pleuropneumonia

Plates 1 – 6. Lung lesions from acute field cases of CCPP
Plate 7. Cross-sections of an acute CCPP lesion
Plate 8. Acute fibrinous pericarditis and epicarditis
Plate 9. Lungs from a chronic field case of CCPP
Satisfactory growth of the Plasmodium was achieved weekly with the
daily subculture. broth cultures appeared in 10 days at
37°C, although colonies only appeared after 5-7 days.

In this way all cultures of Plasmodium have been
subcultured and all results obtained in the broth cultures.

All cultures were transplanted to mice and
eliminated. This procedure was repeated with a rough surface and permanent cages. The parasitic stage
was to be studied in detail.
CULTURAL RESULTS

Acute Cases

Isolation of a mycoplasma related to the M. mycoides species (F38). When studied by dark ground microscopy fresh lesion material from acute field cases nearly always contained numerous branching filaments.

Satisfactory growth of these filaments was achieved using VFG50 and VFG agar. By daily subculture broth cultures attained a titre of $10^7 - 10^8$ cfu/ml, although colonies only appeared after 2-3 days incubation at 37°C. Occasional broth cultures achieved a titre of over $10^9$ cfu/ml.

In this way 41 isolates from 30 field outbreaks of CCPP have been cultured and all were inhibited by growth inhibiting antiserum prepared in rabbits to one isolate, F38. From most outbreaks only 1 case was received for study.

F38 was used for cultural and serological studies as well as for two transmission experiments. Further pathogenicity studies were undertaken with a similar isolate, G69.

Other mycoplasma isolates. From 14 acute cases of CCPP another mycoplasma was isolated. The agent grew more rapidly than the F38 type, formed colonies with a rough surface and fermented glucose. This organism has yet to be studied in detail.

Bacteriological isolates. Of 38 acute cases from 14 outbreaks examined bacteriologically 17 were positive. The isolates were as follows: Pasteurella species, 8 cases; Corynebacterium species, 4 cases; Anthracoids, 4 cases; Streptococcus species, 2 cases; Staphylococcus species, 2 cases; Actinobacillus species, 1 case.
Virus isolation. Five acute CCPP cases from 3 outbreaks were examined virologically. No evidence of virus infection was obtained. The cases all yielded mycoplasma isolates of the F38 type in VFG50 broth and on VFG agar. The tissue cultures did not grow this mycoplasma satisfactorily.

**Chronic Cases**

The isolation of a second mycoplasma related to the M. mycoides species (F30). A mycoplasma was isolated from 3 field cases, F19, F30 and F65C from 2 goat herds which several months previously had suffered from acute outbreaks of CCPP. From one of these herds during the acute phase of the disease 3 cases were culturally examined and F38 alone was isolated. Clinically the acute disease had responded to intramuscular injection of oxytetracycline hydrochloride. Four other chronic cases from these 2 herds were examined but no mycoplasma were isolated.

The cases were emaciated and F19 and F30 showed a high rectal temperature and pneumonic symptoms. Post mortem examinations revealed extensive fibrous adhesions joining affected lung lobes, the costal pleura, and the pericardium. The parietal pleura and pericardium were thickened with fibrous tissue. Between 0.33 and 0.75 of the lung tissue was collapsed and fibrotic, enclosing areas of grey to white necrotic tissue varying from 1 - 2 mm to several cm in diameter. The appearance of the opened chest cavity was similar to Plate 9. In F19 and F65C the affected lung tissue enclosed two 2 - 4 cm diameter heavily encapsulated abscesses containing pus and necrotic tissue.

The lung lesions of F19 and F30 yielded *Pasteurella haemolytica* and *Pasteurella multocida*. Those of the third case, F65C, yielded *Pasteurella haemolytica* alone.
Abundant, rapidly growing mycoplasma were isolated from the lung lesions of all 3 cases. One isolate, F30, was chosen for further study. The other isolates were found to be inhibited by antiserum prepared against F30. The agents grew rapidly on all the mycoplasma media employed in this study. Newing's tryptose media were adopted for routine investigation of these strains.
PATHOGENICITY OF ISOLATE F30

Pathogenicity for Goats

The results of the three experiments with F30 in goats are summarized in Table 8.

Intratracheal endobronchial route of inoculation. Goats inoculated by the intratracheal endobronchial route showed a rise in rectal temperature and depression within 2 - 5 days. Rectal temperatures rose sharply up to 42°C, remaining high or falling 1 - 2 days before death. When a swelling occurred at the site of inoculation this developed by 3 days. Later dyspnoea became marked and a harsh and intermittent cough was noticeable in 2 cases. Affected goats continued to eat with reduced appetite until 1 - 2 days before death. Several cases terminally emitted a continuous groaning noise. Terminally 6 of the affected goats inoculated endobronchially developed mild diarrhoea. All the inoculated goats in the experiment died between 3 and 14 days post inoculation (p.i.), but the in-contact goats remained unaffected.

All infected animals in the intratracheal endobronchial experiment developed lung lesions (see Plates 10, 11 and 12). Four goats suffered severe lung lesions which replaced 0.17 to 0.33 of the total lung tissue. They also had small lesions about the site of entry of the endobronchial tube in the trachea. The other 4 cases showed smaller focal lung lesions but extensive lesions around the trachea (Plates 15 and 16). The latter lesions were similar to those following intramuscular inoculation. In the 4 cases with extensive lung lesions one side of the lung was more severely affected and in 3 of the cases the lesions were bilateral. Affected lung tissue was red to grey, oedematous in section, and
similar in texture to liver tissue; overall the lesions were softer and more oedematous than those seen in field cases. They did not show the elevation, sharply defined edges, or colour mosaic seen in lesions of the natural disease (Plates 10, 11, and 12).

In 2 cases isolated areas with enlarged, oedematous, interlobular septae were observed. In 3 cases the central areas of larger lung lesions were paler, more friable, and somewhat necrotic (Plates 10 and 11). Extensive lung lesions were covered with fibrin varying from slight thickening of the pleura to a yellow gelatinous layer 0.67 cm thick, which joined lung lobes, attached lesions to the costal pleura, and thickened the pericardium (Plates 10 and 11). The chest cavity in these cases contained clear yellow pleural fluid varying from 150 ml to 1.5 litres. In 1 case fibrinous epicardial adhesions and floccules were present in the pericardial sac, which contained excessive clear yellow exudate (Plate 13). A second case showed lesions involving the costal muscles and penetrating to the subcutaneous tissue behind the elbow (Plate 14). These lesions resembled those following intramuscular inoculation of the organism. In 2 affected goats the thymus was enlarged showing prominent septae containing fibrin and oedema fluid. The bronchial and mediastinal lymph nodes from all inoculated goats were grossly enlarged, showing oedema and haemorrhages in section.

Cases which developed mild diarrhoea terminally had congestion in parts of the mucosa of the large intestine with enlargement of the mesenteric lymph nodes. In 5 the spleen was enlarged and in 1 the splenic capsule was thickened with fibrin, showing multiple haemorrhages up to 0.5 cm in diameter.
<table>
<thead>
<tr>
<th>Route of inoculation</th>
<th>No. of goats inoculated</th>
<th>No. of goats affected</th>
<th>No. which died or were killed</th>
<th>No. from which mycoplasma reisolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intratracheal endobronchial</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Contact</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Intratracheal</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Intratracheal endobronchial</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Contact</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 8. Pathogenicity of isolate F30 for goats
The 8 control goats which were inoculated and the 4 contact goats remained healthy. At the end of the experiment these animals were slaughtered and at post mortem examination there were no lesions indicative of pleuropneumonia. Cultures of lung tissue and mediastinal lymph nodes were negative for mycoplasma.

**Intramuscular route of inoculation.** Severe lameness developed within 3 days of the intramuscular inoculation of F30. It was associated with a painful oedematous swelling of the inoculated limb extending downwards from the site of inoculation as far as the fetlock. Otherwise, the symptoms were as described in the preceding experiment with the exception of coughing. Clear yellow oedema fluid containing fibrin distended the subcutaneous connective tissue, muscle, and tendon fascia. The subcutaneous connective tissue, superficial muscle fascia, and tendon fascia showed numerous deposits of yellow fibrin, petechiae, and ecchymoses (Plate 17). Muscle tissue about the needle track and at the site of deposition of the inoculum was pale, almost white, with a necrotic appearance. The muscle around the necrotic focus showed oedematous separation of intramuscular fascial divisions. In Plate 18 four sections of muscle from the affected limb are compared with a similar section from the uninoculated limb (at the right of the photograph). The necrosis and oedema affected the inoculated muscle bundles throughout their length.

In a goat which died 6 days p.i. all oedema fluid was replaced with yellow gelatinous fibrin deposit. In 2 cases the subcutaneous reaction extended forward as far as the elbow on the affected side.

Another of the goats became lame in both hind legs 18 days p.i. 1 week prior to euthanasia. This goat was emaciated, the liver
pale and friable, and the hip joint of the uninoculated limb contained fibrin floccules and an excess of clear exudate.

Seven of the other 8 goats died between 3 and 11 days following inoculation. The remaining animal was severely affected clinically becoming emaciated and developing similar symptoms to other goats inoculated by this route. The animal recovered by the fifth week. Two months p.i. lameness developed and was associated with swelling of the left elbow, which disappeared after a further month. The goat remained healthy, regaining bodily condition.

All the animals showed enlarged popliteal, inguinal, pre-femoral and iliac lymph nodes at post mortem examination. Affected lymph nodes were haemorrhagic and oedematous in section.

The control goats remained healthy throughout the experiment.

All inoculated goats in the infected group of this experiment and the intratracheal endobronchial experiment which died or were slaughtered yielded abundant growth of mycoplasma, culturally and morphologically similar to F30, from the spleen, liver, kidney and lung, in addition to all the lesions.

All specimens from the infected group of the intratracheal endobronchial experiment were negative for bacteria with two exceptions. The spleen of one inoculated animal yielded *E. coli* while the mesenteric lymph node of a second inoculated goat yielded the same organism. Muscle lesion specimens from inoculated goats in the intramuscular experiment were negative for bacteria.

No lesions were observed in the contact goats at slaughter and no mycoplasma were isolated from lung tissue, mediastinal lymph node, or retropharyngeal lymph node.
Lesions following Experimental Inoculation of F30 into Goats

Plates 10 - 12. Pneumonia lesions
Plate 13. Acute fibrinous pericarditis and epicarditis
Plate 14. Visceral pleura of an affected goat
Plate 15. Subcutaneous lesions arising from the site of inoculation
Plate 16. Cross sections of the trachea and tissues of the neck ventral to the site of inoculation
Plate 17. Subcutaneous lesions following intramuscular inoculation of the hind limb
Plate 18. Four cross-sections of affected muscle of the inoculated limb and one section of similar tissue from the uninoculated limb (right)
Contact transmission. Donor goats in the in-contact experiment showed symptoms and lesions similar to affected animals in the intratracheal, endobronchial, and intramuscular experiments, with 2 exceptions. The first, inoculated by the endobronchial route, became lame with an extensive subcutaneous lesion affecting the right thigh, and similar smaller lesions behind each elbow, in addition to small lesions of red consolidation in the lungs. The lesions yielded mycoplasma resembling F30 in pure culture. In the second case an endobronchially inoculated goat was over 4 months pregnant and although no macroscopic lesions were present in the foetus or foetal membranes, mycoplasma resembling F30 were isolated from the foetal fluids, kidney, liver, spleen, and lung.

None of the in-contact goats became clinically affected and no lesions were observed during post-mortem examinations. In addition, no mycoplasma similar to F30 were isolated from specimens of lung, liver, kidney, spleen, bronchial lymph node, mediastinal lymph node, and retropharyngeal lymph node. However all the in-contact goats developed complement-fixing antibodies to F30; titres ranging from 1/16 to 1/64.

Control contact and inoculated goats remained healthy throughout the experiment.

Pathogenicity for Sheep and Cattle

A summary of the results of experiments with F30 in sheep and cattle is presented in Table 9.

Pathogenicity for sheep. Three of the inoculated sheep died between 9 and 17 days p.i.. Two of these developed symptoms and lesions similar to those in goats inoculated with F30 by the endobronchial route. Both showed extensive lesions about the site of inoculation
in the trachea as well as pleuropneumonia. The third had only an extensive tracheal lesion.

No symptoms were observed in the other inoculated sheep although at post-mortem examinations the lungs of 2 sheep revealed chronic lesions, replacing 0.33 to 0.5 of the apical and cardiac lobes of one side, together with extensive fibrous pleural adhesions. The lesions were fibrotic with well-defined margins containing foci of firm, grey to white, necrotic tissue and pus.

The in-contact sheep remained unaffected and no lesions were observed at post-mortem examination. Control inoculated and in-contact sheep remained healthy throughout the experiment.

From the tissues of the fatal cases mycoplasma similar to F30 were isolated from the lung, liver, spleen, and kidney, in addition to the lesions. The organisms were also isolated from the chronic lung lesions of 1 of the killed sheep. No mycoplasma were isolated from lung, liver, spleen, or kidney specimens from other sheep in the infected group. From the chronic lung lesions found in the 2 slaughtered sheep Staphylococcus species, Pseudomonas species, and Corynebacterium pyogenes were isolated. The specimens from the other sheep in the infected group were negative for bacteria.

Pathogenicity for cattle. The calves inoculated via the intratracheal route showed no symptoms and no lesions were observed at post-mortem examinations. No mycoplasma were isolated from lung, liver, spleen, and mediastinal lymph node specimens or from the tissue about the site of inoculation.

The cattle inoculated subcutaneously were similarly unaffected. Post-mortem examinations of 2 calves 28 days p.i. revealed 0.5 to 5 cm diameter areas of mild haemorrhage at the site of subcutaneous
<table>
<thead>
<tr>
<th>Species and route of inoculation</th>
<th>No. inoculated</th>
<th>No. affected</th>
<th>No. which died or were reisolated in extremis</th>
<th>No. from which mycoplasma were isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep Intratracheal</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Contact</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cattle Intratracheal</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>
inoculation, while the prescapular and axillary lymph nodes of that side were somewhat enlarged. In the last 2 cases, killed 103 days p.i., the axillary and prescapular lymph nodes draining the site of inoculation were also enlarged.

With the exception of 1 of the calves killed 103 days p.i., mycoplasma resembling F30 were isolated from the axillary lymph node draining the site of inoculation and in 2 cases from the prescapular lymph node of the inoculated side. None of the calves in either experiment developed complement-fixing antibodies to F30 antigen or *M. mycoides* subspecies *mycoides* (Gladysdale) antigen.

Control animals in both experiments remained healthy.

**Conclusion.** F30 was shown to be highly pathogenic to goats following intratracheal endobronchial and intramuscular inoculation causing fibrinous pleuropneumonia, fibrinous haemorrhagic connective tissue lesions, and necrotising myositis. The agent was recovered from numerous sites within the body suggesting mycoplasmaemia. Goats in contact with experimental pleuropneumonia cases failed to develop disease but developed complement-fixing antibodies to F30.

Following intratracheal inoculation of sheep the organism caused pleuropneumonia and subcutaneous lesions similar to those observed following experimental inoculation of goats. Calves inoculated by the intratracheal and subcutaneous routes remained unaffected and did not develop complement-fixing antibodies to F30. However F30 was reisolated from the axillary and prescapular lymph nodes draining the site of subcutaneous inoculation for as long as 103 days post-inoculation.

Descriptions of some of the F30 experiments have been published *(MacOwan 1976, appendix)*.
PATHOGENICITY OF TYPE CULTURES FOR GOATS

Pathogenicity of PG₃
A summary of the pathogenicity experiments with type cultures is presented in Table 10.

Intratracheal endobronchial route of inoculation. This agent did not cause clinical disease in the 8 inoculated goats which received a high dose of viable organisms via the intratracheal endobronchial route or in the 4 in-contact goats. At post-mortem examination only very small and probably non-specific lung lesions were observed. However PG₃ was reisolated from 5 of the inoculated goats: from lung lesion in 2 cases; from mediastinal and retropharyngeal lymph nodes in 3 cases; and from a tracheal lymph node near the site of inoculation in 2 goats. Specimens of lung or lung lesion, retropharyngeal and bronchial lymph nodes as well as any other lesions were cultured. The cultures of lung, retropharyngeal, mediastinal, and bronchial lymph nodes from the in-contact goats were negative.

Intramuscular route of inoculation. Similarly PG₃ did not cause disease or lesions following intramuscular inoculation of 6 goats. The organism was reisolated from the iliac lymph node of the inoculated limb of one goat. Specimens of inoculated muscle, popliteal and iliac lymph nodes of the inoculated limb, lung, mediastinal and bronchial lymph nodes were cultured.

In neither the intratracheal-endobronchial experiment nor the intramuscular experiment did any of the goats develop complement fixation antibodies to PG₃.

The control goats in both experiments remained healthy.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Route of inoculation</th>
<th>No. inoculated</th>
<th>No. from which mycoplasma were killed in extremis reisolated</th>
<th>No. from which mycoplasma were killed</th>
<th>No. from which mycoplasma were killed</th>
<th>No. which died</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG3</td>
<td>IT-EB</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Contact</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N108</td>
<td>IT-EB</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Contact</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M.G.</td>
<td>IT-EB</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Contact</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 10. Pathogenicity of type cultures of the subspecies M. mycoides for goats.
Pathogenicity of N108

Intratracheal endobronchial route of inoculation. Following intratracheal endobronchial inoculation no clinical symptoms were observed and no specific lesions were present at slaughter. The agent was recovered after post-mortem examination from 6 of the 8 inoculated goats in the following tissues: retropharyngeal lymph node; a tracheal lymph node near the site of inoculation; bronchial lymph node; mediastinal lymph node; lung; and in 2 cases from a small fibrous subcutaneous nodule at the site of inoculation into the trachea. Specimens of lung, retropharyngeal, mediastinal, and bronchial lymph nodes as well as other lesions were cultured. Eleven of the 12 goats including 3 in-contact animals developed complement-fixing antibodies to N108 ranging in titre from 1/16 to 1/256. The in-contact animals remained unaffected and at slaughter no mycoplasma were reisolated from the lung, mediastinal lymph node, bronchial lymph node, or retropharyngeal lymph node.

Intramuscular route of inoculation. Intramuscular inoculation of N108 caused symptoms in 3 out of 6 goats. Two developed swelling of the inoculated limb and lameness for 3–5 days recovering by day 10. One of these goats showed a body temperature of 40.4°C on day 1 only. The third affected goat showed a transient rise in body temperature to 40°C on day 1 but no other symptoms. At post-mortem examinations the first 2 affected goats showed slight thickening of the connective tissue fascia enclosing the inoculated musculature together with slight fibrous infiltration of the muscle tissue. N108 was reisolated from either the popliteal or iliac lymph nodes in the inoculated limb of 3 cases including the 2 which
showed lameness. In addition the agent was recovered from the mediastinal lymph node of a fourth goat. Specimens of inoculated muscle, iliac and popliteal lymph nodes of the inoculated limb, lung, mediastinal and bronchial lymph nodes were cultured in addition to other affected tissues. All the goats developed complement fixation antibodies, the titres ranging from 1/64 to 1/128. The control goats in both experiments remained healthy.

Pathogenicity of the Vom Strain

Intratracheal endobronchial route of inoculation. When the Vom strain was inoculated via the intratracheal endobronchial route all the goats either died or were killed in extremis. Body temperatures rose to 40 - 41°C within 2 - 5 days p.i. remaining elevated for 2 - 6 days when the goats collapsed, showing in 3 cases a subnormal temperature on the day of death or euthanasia. After the rise in body temperature there developed anorexia, severe dyspnoea, abdominal respiration, and weakness followed by persistent groaning and teeth grinding terminally.

At post-mortem examinations all inoculated goats showed foci of red hepatization in the lungs varying from 2 cm in diameter to involvement of all lobes in one half of the lung. The lesions were similar to those observed following intratracheal endobronchial inoculation of F30 into goats. In 1 case the hepatized lesion showed an area with prominent, oedematous interlobular septae (Plate 19). In 4 cases the lesions were covered with yellow fibrin of variable amount forming adhesions to the chest wall, between lobes, and to the pericardium (Plates 20 and 21). Plate 21 shows a paler friable necrotic area surrounded by darker red hepatized tissue. In 5 cases clear yellow pleural fluid was present, varying in volume
from 25 to 200 ml. In 2 other cases a slight excess of clear pericardial fluid was observed. All cases showed enlarged oedematous and haemorrhagic mediastinal lymph nodes. One goat also showed enlargement of the mesenteric lymph nodes and another had fibrinous arthritis of the left acetabulum.

Seven of the 8 inoculated goats developed an extensive oedematous, haemorrhagic and fibrinous lesion of the subcutaneous tissue overlying the trachea at the site of inoculation (Plate 22). The reaction was similar to those caused by F30 at this site.

The Vom strain was reisolated from all inoculated goats in the following sites: lung lesion, pleural fluid where present, bronchial and retropharyngeal lymph nodes, site of inoculation, liver, spleen, and kidney. All the inoculated goats developed a complement fixing antibody titre of 1/16 to 1/32.

The 4 in-contact goats were clinically unaffected and only 1 developed a complement-fixing antibody titre of 1/16. No lesions were observed at post-mortem examination and no mycoplasma were reisolated from the lung, mediastinal and bronchial lymph nodes, or the retropharyngeal lymph nodes.

Intramuscular route of inoculation. The goats inoculated intramuscularly developed a swelling about the inoculation site and lameness within 2 - 3 days p.i.. Four of the goats became febrile, with temperatures of 40 - 41.4°C at this time until death or euthanasia.

In all, 5 goats either died or were killed in extremis 5 - 7 days p.i., the sixth surviving in an emaciated condition until slaughter 6 weeks p.i.. The goats showed similar symptoms and lesions to those observed following intramuscular inoculation
of F30. In addition minor lesions of red collapsed tissue were seen in the lungs of 3 goats at post-mortem examinations.

The Vom strain was reisolated in the 5 acute cases from all affected tissues and other sites including inoculated limb muscle, iliac and popliteal lymph nodes of the inoculated limb, spleen, kidney, liver, mediastinal lymph node, and lung or lung lesion. Three goats developed a complement-fixing antibody titre of 1/16.

Goats inoculated by both the intratracheal endobronchial and intramuscular routes developed a dramatic leucopenia as a result of a sudden reduction in the numbers of lymphocytes and neutrophils (Tables 11–17 and Figure 3).

The control goats in both experiments remained clinically healthy.

Pathogenicity of *M. mycoides* subspecies *mycoides* (Gladysdale)

**Intratracheal endobronchial route of inoculation.** Three of the 8 goats inoculated by the intratracheal endobronchial route had a transient febrile response, with temperatures of 40 – 41.5°C from 2 – 5 days p.i.. At post-mortem examinations 7 of the 8 inoculated goats showed very small foci of red collapse or fibrosis in the lungs. The organism was reisolated in 1 goat from a lung lesion of red collapsed tissue and from the retropharyngeal lymph node while in a second animal it was recovered from the mediastinal lymph node. Specimens cultured included lung or lung lesion, mediastinal, bronchial, and retropharyngeal lymph nodes.

The 4 in-contact goats developed no symptoms or lesions and no mycoplasma were reisolated from the following specimens: lung; mediastinal, bronchial, and retropharyngeal lymph nodes. Neither inoculated nor in-contact goats developed a complement-fixing antibody titre.
Intramuscular route of inoculation. Of the 6 goats inoculated intramuscularly 1 suffered a transient rise of temperature on the fifth day p.i.. This animal and 1 other developed slight lameness for 2 – 3 days during the first week p.i.. *M. mycoides* subspecies *mycoides* (Gladysdale) was not reisolated from inoculated muscle, iliac and popliteal lymph nodes of the inoculated limb, lung, mediastinal and bronchial lymph nodes. The 2 goats which showed symptoms developed a complement-fixing antibody titre of 1/16 to 1/32 to the agent inoculated.

The control goats in both experiments remained clinically unaffected.

Conclusion
The Vom strain was highly pathogenic to goats causing symptoms and lesions similar to those following experimental inoculation of F30. The 4 goats in contact with those affected with fibrinous pleuropneumonia remained unaffected although one developed complement-fixing antibodies.

Following intratracheal endobronchial inoculation strains PG₃ and N108 were found to be avirulent. PG₃ did not give rise to complement-fixing antibodies in contrast to N108. In the N108 experiment 11 out of 12 goats developed complement-fixing antibodies, including 3 out of 4 in contact animals.

*M. mycoides* subspecies *mycoides* (Gladysdale) inoculated by the intratracheal endobronchial route caused transient symptoms in 3 out of 8 goats although none developed complement-fixing antibodies.

Only strain PG₃ was totally avirulent following intramuscular inoculation and the goats did not develop a complement-fixing antibody titre. Strains N108 and *M. mycoides* subspecies *mycoides*
(Gladysdale) caused transient symptoms by this route. N108 gave rise to complement-fixing antibodies in all 6 inoculated goats while only 2 out of 6 goats developed similar antibodies following intramuscular inoculation of M. mycoides subspecies mycoides (Gladysdale).
Lesions following Experimental Inoculation of Strain Vom

Plate 19. Cross-section of a pleuropneumonia lesion showing enlargement of interlobular septae (formalin fixed tissue)

Plate 20. Pleuropneumonia lesion with extensive fibrinous adhesion

Plate 21. Cross-section of a pleuropneumonia lesion showing a large, pale, necrotic focus surrounded by more recent hepatised tissue (formalin fixed tissue)

Plate 22. Cross-section of trachea and tissues of the neck below the site of inoculation (formalin fixed tissue)
Table 11. Vom intratracheal-endobronchial experiment.

Mean ($\bar{x}$) and Standard Deviation (sd) of Packed Cell Volume (P.C.V.), Haemoglobin concentration (Hb) and Red Blood Cells (R.B.C.) during the first week post inoculation.

<table>
<thead>
<tr>
<th>Days post inoculation</th>
<th>Number of observations</th>
<th>P.C.V.</th>
<th>Hb.</th>
<th>R.B.C.</th>
<th>%</th>
<th>gm. per 100ml</th>
<th>x10^6 per cu.mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\bar{x}$</td>
<td>sd</td>
<td>$\bar{x}$</td>
<td>sd</td>
<td>$\bar{x}$</td>
<td>sd</td>
</tr>
<tr>
<td>0</td>
<td>8</td>
<td>41.9</td>
<td>4.7</td>
<td>13.0</td>
<td>0.5</td>
<td>25.1</td>
<td>1.7</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>37.7</td>
<td>4.8</td>
<td>11.8</td>
<td>0.7</td>
<td>22.3</td>
<td>2.7</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>41.5</td>
<td>4.1</td>
<td>11.6</td>
<td>0.7</td>
<td>24.2</td>
<td>1.9</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>38.4</td>
<td>2.9</td>
<td>12.6</td>
<td>1.7</td>
<td>23.5</td>
<td>2.0</td>
</tr>
</tbody>
</table>
Mean ($\bar{x}$) and Standard Deviation (sd) of White Blood Cells (W.B.C.) during the first week post inoculation.

<table>
<thead>
<tr>
<th>Days post inoculation</th>
<th>Number of observations</th>
<th>Total W.B.C. $x10^3$</th>
<th>Neutrophils $x10^3$</th>
<th>Lymphocytes $x10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\bar{x}$</td>
<td>sd</td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>0</td>
<td>8</td>
<td>19.5</td>
<td>3.4</td>
<td>5.6</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>19.0</td>
<td>5.4</td>
<td>7.6</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>10.0</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>9.0</td>
<td>4.9</td>
<td>3.1</td>
</tr>
</tbody>
</table>
Table 13. Vom intratracheal-endobronchial experiment

Mean ($\bar{x}$) and Standard Deviation (sd) of Packed Cell Volume (P.C.V.), Haemoglobin concentration (Hb) and Red Blood Cells (R.B.C.) of in Contact goats.

<table>
<thead>
<tr>
<th>Day</th>
<th>Number of observations</th>
<th>P.C.V. %</th>
<th>Hb. gm. per 100ml</th>
<th>R.B.C. x 10$^6$ per cu. mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\bar{x}$</td>
<td>sd</td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>39.3</td>
<td>1.35</td>
<td>12.7</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>34.7</td>
<td>2.9</td>
<td>11.5</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>41.4</td>
<td>4.1</td>
<td>11.7</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>34.8</td>
<td>5.8</td>
<td>11.2</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>35.5</td>
<td>1.9</td>
<td>11.7</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>33.9</td>
<td>1.6</td>
<td>11.4</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>36.5</td>
<td>1.4</td>
<td>11.7</td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>33.7</td>
<td>3.3</td>
<td>10.8</td>
</tr>
<tr>
<td>16</td>
<td>4</td>
<td>38.4</td>
<td>2.9</td>
<td>12.1</td>
</tr>
<tr>
<td>18</td>
<td>4</td>
<td>38.5</td>
<td>3.7</td>
<td>11.5</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>30.8</td>
<td>2.2</td>
<td>10.3</td>
</tr>
<tr>
<td>22</td>
<td>4</td>
<td>33.5</td>
<td>4.8</td>
<td>10.8</td>
</tr>
</tbody>
</table>

Mean (\(\bar{x}\)) and Standard Deviation (sd) of white Blood Cells (W.B.C.) in Trachoma. L. Pneumoniae.
Table 14. Vom intratracheal-endobronchial experiment

Mean ($\overline{x}$) and Standard Deviation (sd) of White Blood Cells (W.B.C.) of in Contact goats.

<table>
<thead>
<tr>
<th>Day</th>
<th>Number of observations</th>
<th>Total W.B.C. $\times 10^3$</th>
<th>Neutrophils $\times 10^3$</th>
<th>Lymphocytes $\times 10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\overline{x}$</td>
<td>sd</td>
<td>$\overline{x}$</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>15.7</td>
<td>3.2</td>
<td>6.2</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>16.2</td>
<td>5.2</td>
<td>6.3</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>16.3</td>
<td>4.0</td>
<td>5.4</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>13.7</td>
<td>4.8</td>
<td>4.8</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>15.0</td>
<td>5.1</td>
<td>5.4</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>15.0</td>
<td>4.7</td>
<td>5.7</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>16.0</td>
<td>4.4</td>
<td>7.7</td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>14.6</td>
<td>5.3</td>
<td>5.8</td>
</tr>
<tr>
<td>16</td>
<td>4</td>
<td>14.9</td>
<td>4.4</td>
<td>5.8</td>
</tr>
<tr>
<td>18</td>
<td>4</td>
<td>15.9</td>
<td>3.6</td>
<td>6.2</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>12.3</td>
<td>2.9</td>
<td>4.0</td>
</tr>
<tr>
<td>22</td>
<td>4</td>
<td>12.3</td>
<td>3.3</td>
<td>4.6</td>
</tr>
</tbody>
</table>
Table 15. Vom intramuscular experiment

Mean (\( \bar{x} \)) and Standard Deviation (sd) of Packed Cell Volume (P.C.V.), Haemoglobin concentration (Hb) and Red Blood Cells (R.B.C.) in the five fatal cases.

<table>
<thead>
<tr>
<th>Days post inoculation</th>
<th>Number of observations</th>
<th>P.C.V. (% of vol.)</th>
<th>Hb. (gm. per 100 ml.)</th>
<th>R.B.C. (x10^6 per cu.mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \bar{x} )</td>
<td>sd</td>
<td>( \bar{x} )</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>40.7</td>
<td>3.3</td>
<td>13.1</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>36.9</td>
<td>2.5</td>
<td>11.3</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>38.0</td>
<td>2.6</td>
<td>11.0</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>40.4</td>
<td>2.1</td>
<td>12.8</td>
</tr>
</tbody>
</table>
Table 16. Vom intramuscular experiment

Mean ($\bar{x}$) and Standard Deviation (sd) of White Blood Cells (W.B.C.) in the five fatal cases.

<table>
<thead>
<tr>
<th>Days post inoculation</th>
<th>Number of observations</th>
<th>Total W.B.C. $\times 10^3$</th>
<th>Neutrophils $\times 10^3$</th>
<th>Lymphocytes $\times 10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\bar{x}$</td>
<td>sd</td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>19.4</td>
<td>2.2</td>
<td>6.4</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>16.2</td>
<td>4.4</td>
<td>7.4</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>7.8</td>
<td>2.4</td>
<td>2.7</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>6.9</td>
<td>2.3</td>
<td>2.8</td>
</tr>
</tbody>
</table>
Table 17. Vom experimental controls

Mean ($\bar{x}$) and Standard Deviation (sd) of Packed Cell Volume (P.C.V.), Haemoglobin concentration (Hb), Red Blood Cells (R.B.C.) and White Blood Cells (W.B.C.) in 16 Control Goats.

<table>
<thead>
<tr>
<th></th>
<th>P.C.V. %</th>
<th>Hb. gm. per 100 ml</th>
<th>Total R.B.C. x $10^6$ per cu. mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{x}$</td>
<td>35.2</td>
<td>11.5</td>
<td>22.7</td>
</tr>
<tr>
<td>sd</td>
<td>7.0</td>
<td>1.3</td>
<td>3.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Total W.B.C. x $10^3$</th>
<th>Neutrophils x $10^3$</th>
<th>Lymphocytes x $10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{x}$</td>
<td>16.2</td>
<td>4.5</td>
<td>10.6</td>
</tr>
<tr>
<td>sd</td>
<td>3.8</td>
<td>1.7</td>
<td>3.6</td>
</tr>
</tbody>
</table>
VOM EXPERIMENT
MEAN LEUCOCYTE COUNTS

**Lymphocytes**

- Contacts
- i/t
- i/m

**Neutrophils**

- Contacts
- i/t
- i/m

**Total Leucocytes**

- Contacts
- i/t
- i/m

Days Post Inoculation

Figure 2
Pathogenicity of Isolate F38 to Goats

Intratracheal endobronchial inoculation. In the first experiment when high passage culture of F38 was inoculated via the intratracheal endobronchial route all inoculated and in-contact goats remained clinically healthy (Table 18). Only 1 inoculated goat and 1 in-contact goat developed complement-fixing antibody titres of 1/16 and 1/32 respectively.

In the second experiment lower passage culture was used as the inoculum and 3 of the 8 inoculated goats developed acute pleuropneumonia (Table 18). Two cases became febrile for 6 days before death, 16 and 21 days p.i. respectively. Temperatures reached peaks of 41.6°C and 41.7°C, but the goats were alert and active until 2 - 4 days before death when coughing, inappetance, and depression became apparent. The third goat also showed a similar phase, being febrile from day 8 to 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 6 weeks later by which time the animal was chronically affected and emaciated.

Both the acute cases showed fibrinous pleuropneumonia similar to that observed in field cases of CCPP. In Plate 23 the large, pale brown, hepatized lesion showed a grey central area of necrosis and the sharply defined elevated border was clearly visible. Their chest cavities contained 100 - 150 ml of clear yellow pleural fluid. Post-mortem examination of the chronic case revealed extensive fibrotic replacement of lung tissue involving most of the left half of the lung. The fibrous tissue was continuous with and
greatly thickened the pericardium as well as joining the lung to the costal pleura. On incision the fibrotic lesion contained large foci of dry necrotic tissue which still retained some vestige of lung structure in that the larger air passages remained patent.

Two of the in-contact goats became clinically affected, 1 dying 26 days post exposure, the other being killed on day 38. The former remained afebrile, developing a cough and inappetence 2 days before death. At post-mortem examination 0.67 of the left diaphragmatic lobe was replaced by hepatized tissue with a central necrotic area (Plate 24). The lesion had a distinctly blue tinge similar to that commonly seen in field cases when the animal had died some hours previously. There was a thin layer of fibrin covering the central part of the lesion, joining it to the costal pleura.

The goat killed on day 38 became febrile showing anorexia and coughing during the last 9 days. The left lung showed deeply penetrating variegated hepatized lesions affecting the apical and cardiac lobes (Plate 25).

In a third in-contact goat a total of 6 foci, 0.5 - 1 cm diameter, of variegated hepatized tissue were present in the left apical, cardiac, and diaphragmatic lobes, and right cardiac lobe. This was a subclinical case. All affected goats in this experiment showed grossly enlarged and oedematous mediastinal and bronchial lymph nodes.

F38 was reisolated in pure culture from the lung lesions and mediastinal lymph nodes of the 2 inoculated goats which developed fibrinous pleuropneumonia and of the 3 affected in-contact goats.

Inoculated and in contact control goats remained healthy. At the end of the experiment these animals were slaughtered and at post mortem examination there were no lesions indicative of
<table>
<thead>
<tr>
<th>No. of goats inoculated</th>
<th>No. of goats affected</th>
<th>No. which died or were killed in extremis</th>
<th>No. from which mycoplasma were reisolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. IT-EB</td>
<td>7</td>
<td>0</td>
<td>N.A.</td>
</tr>
<tr>
<td>II. IT-EB</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>III. Intravenous</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Key:
- IT-EB: Intratracheal endobronchial
- N.A.: Not applicable

Table 18. Pathogenicity of F38 and G69 isolates for goats.
38 in-contact cases.

Plate 25. An early lesion of pleuropneumonia.
pleuropericardium. In synovia were isolated from the specimens of normal lung tissue.

23

The 4 donor goats were injected intravenously with 20 ml of culture of 669, a broth culture of 009, a culture of MT 68, and a control goat infected with sterile broth to serve as negative control for post-mortem examination. The lungs were hemorrhagic with pleurisy, and small anemias were present. The serums of the infected donor

24

goats. All other goats showed a rise in the serum complement (table 18). The symptoms were similar to those on day 18, the temperature remained elevated, and the goats became similarly affected.

25

The third donor goat showed no change after exposure, remaining normal until death on day 18.

Of the 7 intact goats, 25, 40, and 43 days post exposure died 30, 40, and 43 days post exposure.

The liver of fibrinous pleurisy, pneumonia, and emphysema. The gas in the pleural stage within the emphysematous

26

pleura was not visible. The lungs were slightly opaque, with a grayish discoloration of the pleura.
pleuropneumonia. No mycoplasma were isolated from the specimens of normal lung tissue or mediastinal lymph node.

In-Contact Transmission with a Second F38-Type Isolate (G69)

The 4 donor goats received an initial inoculation of chloroform intravenously which caused unconsciousness for 15 to 30 seconds. Two hours later each goat was injected intravenously with broth culture of G69, a second F38 type isolate.

A control goat inoculated in this way and then inoculated with sterile broth medium intravenously was killed immediately for post-mortem examination. The lungs were haemorrhagic with petechiae and small ecchymoses following the course of the interlobular septae. All other organs appeared normal.

Fourteen days p.i. the first goat inoculated with culture showed a rise in temperature, coughing, and dyspnoea (Table 18). The symptoms were manifested for 8 days prior to euthanasia on day 22, the temperature reaching a peak of 41.4°C. The second goat became similarly affected 39 days p.i. for 9 days prior to slaughter. The third donor goat developed similar symptoms 42 days post exposure, remaining febrile for 9 days, while the fourth remained unaffected.

Of the 7 in-contact goats 5 developed the same symptoms 21, 35, 40, and 42 days post exposure for 6 - 10 days, when they were slaughtered. The remaining 2 showed partial anorexia and died 31 days post exposure without becoming febrile.

The 2 inoculated goats examined post mortem showed lesions of fibrinous pleuropneumonia. The lung lesions were distinguished by the prominence of enlarged grey interlobular septae, not only within the variegated hepatized lesion but throughout the lung.
tissue (Plate 26). Otherwise the lesions were similar to those found in field cases of the disease (Plate 27).

One of the subclinically affected contact cases showed nonspecific lesions at post-mortem examination. The lung was very congested with small areas of red collapsed tissue. The remaining 6 contact cases showed lesions of fibrinous pleuropneumonia with many of the characteristics of lesions seen in field cases of CCPP, for example,

(1) Prominent hepatized lesions (Plates 28 - 33) varying from brown to a mosaic of colours (Plates 31 and 32) and from a soft, liver-like texture (Plates 28 and 33) to a dense and friable consistency (Plates 30 - 32), with only occasional areas showing enlargement of interlobular septae (Plate 34).

(2) Extensive fibrinous pleurisy giving rise to adhesions between lung lobes and the pericardium and attaching lesions to the parietal pleura (Plates 29, 30, and 33). Three cases showed 50 - 100 ml of clear yellow pleural fluid.

(3) The centre of larger lung lesions showed a 'watermark' denoting a more necrotic and friable zone in 3 cases (Plates 28, 31, and 33).

(4) In 3 cases the edge of the lesion was soft and oedematous merging with surrounding unaffected lung (Plates 28, 29, and 33), while in others there was a sharply defined elevated border of firmer hepatized tissue (Plate 31).

(5) The gross enlargement of hepatized lesions was very apparent in one case (Plate 30).

(6) In all cases the mediastinal lymph nodes were enlarged and oedematous.
G69 in Contact Cases

Plates 26 and 27. A case of pleuropneumonia following inoculation of G69, whole lung and sections of lung lesions respectively.

Plates 28 to 33. Six cases of experimental contagious caprine pleuropneumonia.

Plate 34. Cross-section of a pleuropneumonia lesion showing enlargement of interlobular septae.
The mycoplasma inoculated was recovered from the lung lesion, mediastinal lymph node, and pleural fluid where present in all cases except the 1 in-contact goat which showed non-specific lung lesions. In the latter the agent was reisolated from the mediastinal lymph nodes only. The isolates from the 6 specifically affected contact cases were inhibited by hyperimmune serum to F38.

The control goats remained clinically unaffected.

Contact Challenge Experiment with Strains F38 and G69

Three groups of goats were challenged with donor goats which had contracted pleuropneumonia by contact with goats experimentally inoculated with G69 (Table 19). The first group had survived intratracheal endobronchial inoculation with F38 4 months previously while the second group had survived being in contact with these goats. Complement-fixing antibodies had been detected in the sera of one goat in each of the groups. The third group comprised susceptible goats.

Two goats in the previously inoculated group became febrile after contagious challenge but none died (Table 20). Five of the goats in group 2 became febrile and two died while all the susceptible animals in group 3 became febrile and one died.

The probability of developing a fever appeared to be influenced by the pre-challenge treatment; chi-square = 6.562 (P<0.05) when computed by Snedecor and Irwin's method (Snedecor, 1956). The difference appeared to be attributable to the low proportion of animals in the first or previously inoculated group; when the first and second groups were assessed by Fisher's 'exact test' (Fisher, 1958) the probability was 12.1 per cent but the first and third groups yielded a probability of 4.5 per cent. Moreover, when the
first group was assessed against a pool of the second and third groups the probability was 2.7 per cent. The second and third groups yielded a probability of 54.4 per cent.

The onsets of the febrile phase were not significantly different in groups 2 and 3 \( (t = 0.2446(8), P > 0.50) \). Similarly the duration of fever was not significantly different between these two groups \( (t = 0.174(8), P > 0.50) \).

To continue contagious challenge of groups 1, 2, and 3 another 2 groups of susceptible goats, groups 4 and 5, were introduced after goats in group 3 became febrile. All the goats in groups 4 and 5 developed disease (Table 21).

Minimal incubation periods in cases infected by contact ranged from 11 to 21 days. The febrile phases of 18 contagious cases were pooled to establish the norm. (Table 22, Figure 4). Fevers above 40°C persisted for 8 days, peak temperatures of 41.3 and 41.2°C being attained on the second and third febrile days.

Goats which survived the febrile phase were slaughtered at an increasing time interval after their rectal temperatures returned to normal.

At post-mortem examination the 4 cases which died showed lesions similar to those described for 6 out of 7 in-contact cases in the contact experiment with G69. With the exception of goats 1 and 2, the goats in Group 1 showed no lesions (Table 19). In goat 1 the left apical lobe, 0.5 of the left cardiac lobe and the anterior dorsal 0.3 of the right diaphragmatic lobe were replaced with dense yellow necrotic tissue enclosed within a thick fibrous capsule which had a narrow margin of red collapsed tissue in parts.
In addition the right cardiac lobe was joined to the right diaphragmatic lobe by fibrous tissue. The mediastinal lymph nodes were somewhat enlarged and oedematous. In the second goat the left diaphragmatic lobe was totally replaced with an acute lesion similar to those described in 6 out of 7 contact cases in the preceding experiment. This lesion was covered with yellow fibrin and adherent to the chest wall. 100 ml of clear yellow pleural fluid was found in the left chest cavity. The right lung showed fibrous adhesion between the apical and cardiac lobes and the pericardium. The mediastinal lymph nodes were grossly enlarged and oedematous. This goat showed a biphasic febrile reaction 28 and 57 days post exposure lasting for 4 and 2 days respectively.

In Group II goat number 6 showed no lesions at post mortem examination while the three other goats killed showed extensive chronic lesions similar to those of the first goat in Group I. Goats 3, 4, and 5 in Group 3 also showed this type of lung lesion at post mortem while the first goat of this group killed only eight days after the febrile phase showed the extensive acute lesion seen in the specifically affected contact cases of the preceding experiment.

Those goats killed more than eight days after the febrile phase did not have filaments readily visible by dark ground microscope in wet preparations of macerated lung lesion. The organism was reisolated from lung lesion and mediastinal lymph node of an affected goat 33 days after the febrile phase (Table 23). Two goats in Group 1 which did not show a febrile response harboured the organism in the retropharyngeal lymph nodes, although no abnormality or mycoplasma were found in the lung or mediastinal lymph nodes of these goats.
Table 19. Contact challenge experiment with strains F38 and G69

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of goats</th>
<th>Previous treatment</th>
<th>Goat</th>
<th>Febrile phase</th>
<th>Killed or Died</th>
<th>No. days died/affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>F38</td>
<td>1</td>
<td>15 - 21</td>
<td>Killed D31</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IT-EB</td>
<td>2</td>
<td>28 - 31</td>
<td>&quot; D61</td>
<td>57 - 58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td>Killed D62</td>
<td>2/6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td></td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td></td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>F38</td>
<td>1</td>
<td>20 - 26</td>
<td>Died D27</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contact</td>
<td>2</td>
<td>20 - 27</td>
<td>Killed D42</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>20 - 28</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>20 - 28</td>
<td>&quot; &quot; 5/6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>27 - 30</td>
<td>Died D32</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td></td>
<td>Killed D62</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>Nil</td>
<td>1</td>
<td>15 - 20</td>
<td>Killed D28</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>18 - 25</td>
<td>Died D30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>20 - 25</td>
<td>Killed D58</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>27 - 35</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>31 - 39</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
</tbody>
</table>

**Key:** IT-EB : intratracheal endobronchial  
D : day
<table>
<thead>
<tr>
<th>Group</th>
<th>Previous treatment</th>
<th>No. of goats</th>
<th>No. febrile</th>
<th>No. which died</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F38 IT-EB</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>F38 contact</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Nil</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 20. Results of contact challenge experiment with strains F38 and 069
Table 21. Contact challenge experiment with strains P38 and G69: Goats introduced to continue contagious challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of goats</th>
<th>Previous treatment</th>
<th>Goat no.</th>
<th>Febrile phase</th>
<th>Died</th>
<th>No. affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3</td>
<td>Nil</td>
<td>1</td>
<td>12 - 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>13 - 21</td>
<td></td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>17 - 25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>Nil</td>
<td>1</td>
<td>11 - 14 &amp; 23</td>
<td></td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>15 - 18 and 22 - 23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>15 - 18</td>
<td></td>
<td>D21</td>
</tr>
<tr>
<td>Days post febrile</td>
<td>No. of observations</td>
<td>Temperature $^\circ C$</td>
<td>$\bar{x}$</td>
<td>sd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>---------------------</td>
<td>-----------------------</td>
<td>----------</td>
<td>----</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-7</td>
<td>18</td>
<td>38.3</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-6</td>
<td>18</td>
<td>38.3</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-5</td>
<td>18</td>
<td>38.2</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-4</td>
<td>18</td>
<td>38.4</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-3</td>
<td>18</td>
<td>38.2</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2</td>
<td>18</td>
<td>38.6</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1</td>
<td>18</td>
<td>39.2</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>40.8</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>41.3</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>41.2</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>40.9</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>40.4</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>17</td>
<td>40.3</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>40.2</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>40.1</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>13</td>
<td>39.9</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td>39.3</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>11</td>
<td>39.3</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>39.1</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>9</td>
<td>38.8</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>8</td>
<td>38.6</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>38.7</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FEBRILE PHASE OF EXPERIMENTAL CONTACT CASES

Figure 3
Table 23. Cultural results

<table>
<thead>
<tr>
<th>Group</th>
<th>Goat</th>
<th>Days post febrile until death or slaughter</th>
<th>Mycoplasma re-isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lung</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mediastinal lymph node</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Retropharyngeal lymph node</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>8</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>14</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>19</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>23</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>33</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

Key: + : Mycoplasma isolated
- : No mycoplasma isolated
Experimental Control Goats

The control goats in the intratracheal endobronchial experiment with F30 and the second experiment with F38 were killed. No lesions were found in the lungs of these 24 goats and specimens of lung tissue cultured for mycoplasma were negative.

While the control goats in the other experiments were not killed they remained clinically unaffected.

All 36 inoculated but unaffected goats in the infected groups of the FG3, N108 and M. mycoides subspecies mycoides (Gladysdale) experiments were additional controls for the second experiment with F38 and for the G69 experiment. The goats in these experiments came from the same herd and they were of similar age.

A G69 intratracheal endobronchial contact experiment with stringent controls

All inoculated goats in the infected group became febrile 3 - 9 days post-inoculation (p.i.) and developed clinical signs of pneumonia. Six reacted severely with peak temperatures of 41°C or more, temperatures remaining over 40°C for 5 - 10 days. The other 4 inoculated goats developed similar symptoms but peak temperatures of 40.7°C or less, temperatures remaining over 40°C for 2 - 5 days. All inoculated goats were killed 5 - 10 days after their body temperatures returned below 40°C.

The 10 in-contact goats in the infected group reacted similarly between 19 and 30 days post exposure and all developed peak temperatures of 41°C or more. These cases were killed during the febrile phase.

At post mortem examination all inoculated and in-contact goats had CCPP lesions similar to those described in the second F38 experiment and in the in contact transmission experiment with isolate
The lesions of each in-contact case and 1 inoculated case are illustrated (plates 35-45). Lesions in 4 of the 10 inoculated cases showed signs of early resolution. Fibrin when present was dry and firm while lesions were friable even at the periphery, and no pleural fluid was present.

Mycoplasma were isolated in all contact cases from the lung lesions and the mediastinal lymph nodes, in 8 cases from the tracheobronchial lymph nodes, in 7 cases from the pleural fluid and in one case from the retropharyngeal lymph node. From 6 inoculated cases mycoplasma were isolated in 5 cases from lung lesion, in 2 cases from fibrin and in 1 case from mediastinal lymph node. One isolate from each of the 16 cases which yielded mycoplasma was tested by growth inhibition test with antiserum prepared in rabbits to F38. All isolates were totally inhibited.

Other than Pasteurella haemolytica var haemolytica and Corynebacterium pyogenes from the lung lesion of one inoculated case no bacteria were isolated. Parainfluenza III virus (PI3) was isolated from the lung lesion specimen of one in-contact case.

None of the control goats developed any clinical signs and at post-mortem examination no lesions indicative of pleuropneumonia were observed. No mycoplasma or bacteria were isolated in any specimens from control animals. PI3 virus was isolated from a retropharyngeal lymph node specimen of one inoculated control goat. Blood samples taken from all goats prior to the experiment yielded haematological results within normal limits. (Schalm, Jain and Carrol, 1975).

Sera taken from all goats prior to the experiment did not
react with F38 antigen by complement fixation test. A titre of 1/16 or less was considered negative. Similarly sera from all control goats remained negative. The sera of all inoculated goats in the infected group became positive to a high titre (Table 24), but sera from only 2 in-contact cases became positive. A titre of 1/32 or more was considered positive. One in-contact case, febrile for 9 days, developed a titre of 1/512 and the other, febrile for 5 days, showed a titre of 1/128. The remaining in-contact cases were killed during their febrile phase, after 4 - 6 days of fever, and they did not develop titres higher than 1/16.

An experiment to enable further serological study of G69 contact cases.

All 8 in-contact goats developed symptoms similar to those in the previous experiment 19 to 66 days post exposure. The febrile phase ranged from 3 to 10 days. Two goats died during the febrile phase on the 5th and 9th day of fever respectively, while a 3rd case died 1 day after body temperature fell below 40°C. These 3 goats showed lesions similar to those described previously for G69 contact cases and mycoplasma were reisolated from the lung lesion and mediastinal lymph nodes of each case. A mycoplasma isolate from each case was inhibited in the growth inhibition test by hyperimmune rabbit serum prepared to strain F38.

All but 1 case developed complement-fixing antibodies to strain F38 between the 3rd and 7th day of fever. The negative case died on the 5th day of fever. Peak titres ranged from 1/256 to 1/2048.

Conclusion

F38 and F38-type isolate G69 were shown to be pathogenic to goats causing a highly contagious pleuropneumonia with a minimum incubation period of 11 - 21 days. The experimental disease spread
contagiously not only from inoculated donor goats to in-contact susceptibles, but also from contact cases to susceptibles. Of 46 susceptible goats challenged by contact 43 became febrile and showed pneumonic symptoms.

At post-mortem examination acute pleuropneumonia was present in 26 contact cases and chronic pleuropneumonia lesions arising from contact cases were observed in 8 goats.

F38 was reisolated from all acute contagious cases which died or were killed. Reisolates from 20 acute contact cases were confirmed to be of the F38 type by growth inhibition test using hyperimmune serum prepared to F38 in rabbits. F38 was reisolated from 2 in-contact experimental goats as long as 62 days post exposure. Both inoculated and in-contact cases developed complement fixing antibodies to F38 antigen.

Prior contact exposure of goats to goats inoculated with F38 did not apparently affect their susceptibility to contagious infection by goats inoculated with F38-type isolate G69. In contrast prior inoculation of goats with high passage F38 broth culture appeared to afford a measure of protection from contagious pleuropneumonia.

Descriptions of the second intratracheal endobronchial experiment with F38 and an experiment with G69 are in press (MacOwan and Minette, 1976).
Table 24  G69 intratracheal endobronchial contact experiment

Complement fixation test - Inoculated goats in the infected group.

<table>
<thead>
<tr>
<th>Goat no.</th>
<th>Onset of fever Day</th>
<th>Days febrile post</th>
<th>Days febrile until death</th>
<th>Days bled post inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>8</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>2</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>10</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>5</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>8</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>9</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>5</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Key: Serum titres = reciprocals of the 50% end point dilutions.

*: Goats killed.
Lung lesions from cases in the C69 intratracheal endobronchial experiment with stringent controls.

Plate 35. Lung lesion following inoculation of C69

Plates 36-45. Lung lesions of in contact cases.
SEROLOGICAL RESULTS

Serological Comparison of F30 and F38 with Reference Organisms of the M. mycoides Species

Serological Studies with Reisolates, Lesion Material and Sera from Experimental Cases

Growth inhibition test. All reisolates in the G69 experiments were inhibited by growth-inhibiting rabbit serum prepared to F38. In the contact challenge experiment with strains F38 and G69 reisolates were not tested in this way.

In the PG³ intramuscular experiment and the M. mycoides subspecies mycoides (Gladysdale) intratracheal endobronchial experiment all mycoplasma reisolated were inhibited by their homologous rabbit growth inhibiting serum. In the other experiments only reisolates from 3 cases in each experiment were tested and all were found to be inhibited by their homologous rabbit growth inhibiting serum (Table 25).

Agar gel double diffusion test. Lung lesion material from 2 goats inoculated with F30 and a subcutaneous lesion from 1 goat similarly inoculated reacted with rabbit hyperimmune sera to all the reference strains and F30. Lung lesion from 3 sheep similarly inoculated also reacted to all these hyperimmune sera (Table 26).

Lung lesion from 2 cases inoculated with F38 reacted with hyperimmune rabbit sera to all the reference strains and F30. In addition lung lesion from 3 in-contact cases reacted in this way, that is 2 cases reacted to all these sera with the exception of those to Vom and Smith strains, while the lung lesion of 1 goat reacted only to PG³ and Smith hyperimmune sera. In all, lung
lesion material from 15 in-contact cases was tested. The last sera before death or euthanasia from 12 in-contact cases in these 2 experiments reacted with none of the concentrated antigens to the reference strains, F30, or F38.

Sera from 2 inoculated goats in the infected group of the G69 intratracheal endobronchial experiment formed a common line with antigens of strains Vom, PG3, M. Mycoides subspecies mycoides, F30 and F38. Serum samples taken from these 2 goats prior to the experiment did not react with antigens to the reference strains, F30, or F38.

Both positive antisera and their preinoculation sera were concentrated to half their volume and dialysed, the preinoculation sera remained negative while the post-inoculation sera gave stronger homologous and heterologous reactions. These antisera showed 3 lines with F38 antigen, 1 of which was common to M. mycoides subspecies mycoides, PG3 and Vom antigens, while a second line was common to M. mycoides subspecies mycoides and PG3 antigens only.

Complement fixation test. The last sera before death or euthanasia from 15 experimental F38 and G69 contact cases were tested against both F38 and F30 antigens. All these sera were positive to both antigens, titres ranging from 1/16 - 1/256. There was little difference in the titres of any serum when reacted with either antigen.

Serological Studies with Lesion Material and Sera from Field Goats

Agar gel double diffusion tests. Pleural fluid and/or lung lesion from 39 field cases from 22 outbreaks were tested against hyper-immune rabbit sera prepared to F30, PG3, M108, Vom, Smith, and M. mycoides subspecies mycoides (Gladysdale) strains (Table 27).
Lung lesion material from 13 out of 39 cases from 7 out of 22 outbreaks reacted with all 6 hyperimmune sera.

All the hyperimmune rabbit antisera, including hyperimmune serum to F38, also reacted with lung lesion from a field case of contagious bovine pleuropneumonia. The antisera did not react with normal bovine lung or serum.

Sera from goats in herds affected with CCPP were tested against antigens prepared from cultures of F38, F30, NL08, PG3, Vom, Smith, and M. mycoides subspecies mycoides (Gladysdale) (Table 28). CCPP was confirmed at post-mortem examination in at least 1 clinical case from each outbreak.

Five out of 26 sera from 3 out of 9 outbreaks reacted with all antigens.

Complement fixation tests. In the herd from which F30 was isolated from the chronic lung lesions of 2 goats, 21 paired sera from different goats were examined by complement fixation tests using F30 and F38 antigens. F38 antigen detected 21 out of 21 goats with titres ranging from 1/16 - 1/256, while F30 antigen detected 12 out of 21 goats positive, titres varying from 1/16 - 1/64. No rising titres were detected by F38 antigen, while 5 goats showed a greater than fourfold change in titre to F30 antigen.

The results of complement fixation tests with other field goat sera from herds affected with CCPP using F30 and F38 antigens are presented in Table 29. A serum titre of 1/16 or more was considered positive. F38 antigen detected 22 out of 41 goats positive from 10 out of 11 outbreaks, while F30 detected 23 out of 40 goats positive from 9 out of 10 outbreaks. 19 sera from 9 out of 10 outbreaks reacted to both antigens.
Table 25. Growth inhibition tests with experimental reisolates

<table>
<thead>
<tr>
<th>Experiment and route of inoculation</th>
<th>No. of cases studied</th>
<th>No. of reisolates</th>
<th>Growth inhibiting antiserum</th>
<th>No. of reisolates inhibited</th>
</tr>
</thead>
<tbody>
<tr>
<td>F30</td>
<td>3</td>
<td>3</td>
<td>F30</td>
<td>3</td>
</tr>
<tr>
<td>IT-EB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IM</td>
<td>3</td>
<td>3</td>
<td>F30</td>
<td>3</td>
</tr>
<tr>
<td>PG3</td>
<td>3</td>
<td>3</td>
<td>PG3</td>
<td>3</td>
</tr>
<tr>
<td>IT-EB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IM</td>
<td>1</td>
<td>1</td>
<td>PG3</td>
<td>1</td>
</tr>
<tr>
<td>N108</td>
<td>3</td>
<td>3</td>
<td>N108</td>
<td>3</td>
</tr>
<tr>
<td>IT-EB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IM</td>
<td>3</td>
<td>3</td>
<td>N108</td>
<td>3</td>
</tr>
<tr>
<td>Vom</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IT-EB</td>
<td>3</td>
<td>3</td>
<td>Vom</td>
<td>3</td>
</tr>
<tr>
<td>IM</td>
<td>3</td>
<td>3</td>
<td>Vom</td>
<td>3</td>
</tr>
<tr>
<td>MG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IT-EB</td>
<td>2</td>
<td>2</td>
<td>MG</td>
<td>2</td>
</tr>
<tr>
<td>F38</td>
<td>3</td>
<td>3</td>
<td>F38</td>
<td>3</td>
</tr>
<tr>
<td>IT-EB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G69 contact transmission</td>
<td>7</td>
<td>7</td>
<td>F38</td>
<td>7</td>
</tr>
<tr>
<td>G 69</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IT-EB</td>
<td>16</td>
<td>16</td>
<td>F38</td>
<td>16</td>
</tr>
</tbody>
</table>
Table 26. Agar gel double diffusion tests with experimental lesion material

<table>
<thead>
<tr>
<th>Experiment and route of inoculation</th>
<th>No. of cases and lesion studied</th>
<th>Hyperimmune sera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F30</td>
</tr>
<tr>
<td><strong>F30</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IT-EB goats</td>
<td>2/2 lung</td>
<td>+</td>
</tr>
<tr>
<td>IN goats</td>
<td>1/1 C.T.</td>
<td>+</td>
</tr>
<tr>
<td>IT sheep</td>
<td>3/3 lung</td>
<td>+</td>
</tr>
<tr>
<td><strong>F38</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IT-EB goats</td>
<td>2/5 lung</td>
<td>+</td>
</tr>
<tr>
<td>Contact, goats</td>
<td>2/15 lung</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1/15 lung</td>
<td>-</td>
</tr>
</tbody>
</table>

**Key:** C.T.: connective tissue lesion
Table 27. Agar gel double diffusion results using lesion material from field cases as antigen

<table>
<thead>
<tr>
<th>Result</th>
<th>Hyperimmune rabbit antisera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F30</td>
</tr>
<tr>
<td>CCPP cases +ve</td>
<td>18/39</td>
</tr>
<tr>
<td>Outbreaks of CCPP +ve</td>
<td>10/22</td>
</tr>
<tr>
<td>Reaction with goat serum (control)</td>
<td>-</td>
</tr>
<tr>
<td>Reaction with normal goat lung (control)</td>
<td>-</td>
</tr>
</tbody>
</table>

*M.G.: M. mycoides subspecies mycoides (Gladysdale)*
Table 28. Agar gel double diffusion results using sera from field goats

<table>
<thead>
<tr>
<th>Results</th>
<th>Antigen</th>
<th>F38</th>
<th>F30</th>
<th>N108</th>
<th>P03</th>
<th>Vom</th>
<th>Smith</th>
<th>M.G.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sera +ve</td>
<td></td>
<td>13/26</td>
<td>9/26</td>
<td>5/26</td>
<td>9/26</td>
<td>6/26</td>
<td>9/26</td>
<td>7/26</td>
</tr>
<tr>
<td>Normal goat serum</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*M.G. : M. mycoides subspecies mycoides (Gladysdale)*
### Table 29. Complement fixation test results using sera from field goats

<table>
<thead>
<tr>
<th>Outbreak Goats studied to F38 antigen</th>
<th>Sera +ve to F38 antigen</th>
<th>Titres to F38 antigen</th>
<th>Titres to F30 antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>64-512</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>2</td>
<td>64-512</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>3</td>
<td>64-256</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>1</td>
<td>16-32</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>1</td>
<td>16-32</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>3</td>
<td>64</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td>11</td>
<td>16</td>
<td>7</td>
<td>16-128</td>
</tr>
</tbody>
</table>

**Key:** The serum titres are reciprocals of the numbers in the last two columns.

a/c: serum anticomplementary
Serological Studies with Concentrated Antigens and Hyperimmune Sera Prepared in Rabbits

Agar gel double diffusion test. F30 hyperimmune serum prepared by the method of Lemcke (1965) gave only 1 precipitation line with the homologous antigen and no cross reaction with \textit{M. mycoides} subspecies \textit{mycoides} (Gladysdale), in contrast to the method of Krauss and Wandera (1970). The latter method was employed for the preparation of all hyperimmune sera used in agar gel diffusion, complement fixation, and growth precipitation tests in this study.

It was found that antigens which were frozen and then thawed rapidly between 6 and 10 times gave more precipitation lines than untreated antigens. In addition further freezing and thawing resulted in gradual loss of definition of precipitation lines (Plate 46). The total number of lines in each homologous reaction was determined by reacting the antiserum, in the centre well, with at least 3 outer wells of antigen (Plate 47).

The agar gel double diffusion test results indicated the reference strains and F30 were very closely related. A minimum of 2 and a maximum of 5 precipitation lines were observed to be totally or partially common to each strain (Table 30).

Complement fixation tests. Comparison of F30, F38, and reference organisms of the species \textit{M. mycoides} enabled \textit{M. mycoides} subspecies \textit{mycoides} (Gladysdale) to be distinguished from the caprine strains. However high titre cross-reactions were found amongst the caprine \textit{mycoplasma} strains (Table 31).

Sera from 2 cattle with contagious bovine pleuropneumonia were tested against PG\textsubscript{3}, N108, F30, and \textit{M. mycoides} subspecies \textit{mycoides} (Gladysdale). Each serum showed a titre of 1/256 with \textit{M. mycoides} subspecies \textit{mycoides} (Gladysdale) but the heterologous reactions did not exceed 1/16.
Tubc agglutination test. This test was discontinued because antigens autoagglutinated. Even rapid freezing and thawing from 1 to 20 times did not reduce autoagglutination.

Growth precipitation test. The results of growth precipitation supported the agar gel diffusion and complement fixation test findings, except that F38 culture did not react with hyperimmune sera to the reference strains or to F30 (Table 32).

Growth and Metabolic Inhibition Tests. The growth inhibition test separated the strains tested into 3 groups: group 1, F30, Vom and *M. mycoides* subspecies *mycoides* (Gladysdale); group 2, PG3, N108 and Smith; group 3, F38 (Table 33). The metabolic inhibition test showed a similar relationship between these mycoplasma (Table 34).
Acar Gel Diffusion Slides

Plate 46
Well 2, 4, 6: PG₃ antigen
Well 1: F30 antigen
Well 3: N108 antigen
Well 5: M. mycoides subspecies mycoides (Gladysdale) antigen
Centre well: PG₃ antiserum

Plate 47
Well 1, 2, 3, 4: M. mycoides subspecies mycoides (Gladysdale) antigen
Centre well: M. mycoides subspecies mycoides (Gladysdale) antiserum
Table 1a. Effect of Surface Diffusion-Test Reaction

<table>
<thead>
<tr>
<th>Antigen</th>
<th>X10</th>
<th>X50</th>
<th>X100</th>
<th>X500</th>
<th>X1000</th>
<th>X5000</th>
</tr>
</thead>
<tbody>
<tr>
<td>F30</td>
<td>4+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>Von</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>PC3</td>
<td>4-3</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>2108</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>Ca</td>
<td>4-3</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>F.D.</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
</tr>
</tbody>
</table>

Key: Numbers refer to the

- The number of precipitin lines
- The intensity of the precipitin lines
- The reaction condition

- 'X' refers to the concentration of the antigen.

- 'No precipitation' means no reaction.

Z-A. E. M. shows an electrophoretic mobility (E.M. value).

St. Smith strain

P.R. Presumed related medium

F. S. Derived from unincubated medium.
### Table 30. Agar gel double diffusion test results

<table>
<thead>
<tr>
<th>Antigens</th>
<th>F30</th>
<th>Vom</th>
<th>PG3</th>
<th>M108</th>
<th>Sm</th>
<th>M.G.</th>
<th>F.D.</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>F30</td>
<td>4-6</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2-3s</td>
<td>4s</td>
<td>3s</td>
<td>3s</td>
<td>4s</td>
<td>4s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vom</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2s</td>
<td>2s</td>
<td>4-5s</td>
<td>2s</td>
<td>2s</td>
<td>2s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG3</td>
<td>4-5</td>
<td>2</td>
<td>4-5</td>
<td>5</td>
<td>2</td>
<td>3-5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3s</td>
<td>2s</td>
<td>4-5s</td>
<td>1s</td>
<td>3s</td>
<td>3s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M108</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>5-6</td>
<td>2</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4s</td>
<td>2s</td>
<td>4s</td>
<td>1s</td>
<td>4s</td>
<td>4s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sm</td>
<td>4-5</td>
<td>2</td>
<td>3-4</td>
<td>5</td>
<td>4-5</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3s</td>
<td>2s</td>
<td>2s</td>
<td>4-5s</td>
<td>2s</td>
<td>2s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.G.</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>4-5</td>
<td>2</td>
<td>4-6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4s</td>
<td>2s</td>
<td>3-4s</td>
<td>4s</td>
<td>2s</td>
<td>2s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F38</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2s</td>
<td>2s</td>
<td>3s</td>
<td>2s</td>
<td>3s</td>
<td>2s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.D.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Key:** Numbers refer to the number of precipitation lines.

- **s:** The number of precipitation lines totally or partially common to the homologous reaction.

- **-:** No precipitation lines observed.

- **M.G.:** *M. mycoides* subspecies *mycoides* (Gladysdale)

- **Sm:** Smith strain

- **F.D.:** Freeze-dried medium

- **S:** Sediment from uninoculated medium
Table 31. Complement fixation test results

<table>
<thead>
<tr>
<th>Antigen</th>
<th>F30</th>
<th>Vom</th>
<th>M.G.</th>
<th>FG₃</th>
<th>N108</th>
<th>Smith</th>
<th>F38</th>
<th>F.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F30</td>
<td>512</td>
<td>512</td>
<td>64</td>
<td>128</td>
<td>256</td>
<td>2048</td>
<td>256</td>
<td>-</td>
</tr>
<tr>
<td>Vom</td>
<td>1024</td>
<td>512</td>
<td>256</td>
<td>256</td>
<td>512</td>
<td>1024</td>
<td>256</td>
<td>-</td>
</tr>
<tr>
<td>M.G.</td>
<td>1024</td>
<td>512</td>
<td>2048</td>
<td>1024</td>
<td>1024</td>
<td>1024</td>
<td>256</td>
<td>-</td>
</tr>
<tr>
<td>FG₃</td>
<td>512</td>
<td>256</td>
<td>128</td>
<td>2048</td>
<td>2048</td>
<td>2048</td>
<td>256</td>
<td>-</td>
</tr>
<tr>
<td>N108</td>
<td>128</td>
<td>1024</td>
<td>64</td>
<td>256</td>
<td>2048</td>
<td>8192</td>
<td>256</td>
<td>-</td>
</tr>
<tr>
<td>Smith</td>
<td>1024</td>
<td>512</td>
<td>256</td>
<td>512</td>
<td>1024</td>
<td>8192</td>
<td>256</td>
<td>-</td>
</tr>
<tr>
<td>F38</td>
<td>1024</td>
<td>256</td>
<td>256</td>
<td>256</td>
<td>512</td>
<td>1024</td>
<td>512</td>
<td>-</td>
</tr>
<tr>
<td>F.D.</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>16</td>
<td>128</td>
</tr>
</tbody>
</table>

Key: F.D.: Freeze-dried medium antigen

M.G.: M. mycoides subspecies mycoides (Gladysdale)

Numbers: The reciprocal of the numbers represent the antiserum titre
Table 32. Growth precipitation test results

<table>
<thead>
<tr>
<th>Culture</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F30</td>
</tr>
<tr>
<td>F30</td>
<td>+</td>
</tr>
<tr>
<td>Vom</td>
<td>+</td>
</tr>
<tr>
<td>M.G.</td>
<td>+</td>
</tr>
<tr>
<td>Smith</td>
<td>+</td>
</tr>
<tr>
<td>PG</td>
<td>+</td>
</tr>
<tr>
<td>N108</td>
<td>+</td>
</tr>
<tr>
<td>F38</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: + : 1 or more precipitation lines

M.G. : *M. mycoides* subspecies *M. mycoides* (Gladysdale)

N.D. : not done
Table 33. Growth inhibition test results

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>F30</th>
<th>Vom</th>
<th>M.G.</th>
<th>PG₃</th>
<th>N108</th>
<th>Smith</th>
<th>F38</th>
</tr>
</thead>
<tbody>
<tr>
<td>F30</td>
<td>+</td>
<td>+</td>
<td>+wk</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vom</td>
<td>+</td>
<td>+</td>
<td>+wk</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M.G.</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PG₃</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>N108</td>
<td>-</td>
<td>-</td>
<td>+wk</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Smith</td>
<td>-</td>
<td>-</td>
<td>+wk</td>
<td>+wk</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F38</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: M.G. : *M. mycoides* subspecies *mycoides* (Gladysdale)
+wk : weak positive reaction, zone of inhibition much smaller than in the homologous reaction
Table 34. Metabolic Inhibition Test Results

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antiserum</th>
<th>F30</th>
<th>Vom</th>
<th>M.G.</th>
<th>PG</th>
</tr>
</thead>
<tbody>
<tr>
<td>F30</td>
<td>Vom</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.G.</td>
<td>PG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Smith</th>
<th>Vom</th>
<th>F30</th>
<th>Vom</th>
<th>M.G.</th>
<th>PG</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.G.</td>
<td>PG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smith</td>
<td>Vom</td>
<td>F30</td>
<td>Vom</td>
<td>M.G.</td>
<td>PG</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Smith</th>
<th>Vom</th>
<th>M.G.</th>
<th>PG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vom</td>
<td>Smith</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vom</td>
<td>F30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.G.</td>
<td>PG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Key:** M.G.: *M. mycoides* subspecies *mycoides* (Gladysdale)  
*W. N.*: *W. N.*  
W. INO: *W. INO*

Serum titres are the reciprocal of the end point dilution.

Smith, M.G., and PG titres are the reciprocal of the end point dilution.
CULTURAL COMPARISON OF F30, F38, AND REFERENCE STRAINS OF THE SPECIES M. MYCOIDES

Growth

F30 as well as the reference strains, FG3, N108, Vom, Smith, and M. mycoides subspecies mycoides (Gladysdale) grew well in Newing's tryptose medium. Cultures of the caprine strains reached titres of $10^8 - 10^9$ cfu/ml within 24 - 28 hours even when only a few organisms were inoculated. M. mycoides subspecies mycoides (Gladysdale) was slightly slower, achieving similar titres after 48 - 72 hours. Colonies of the caprine strains were visible after 24 hours incubation while M. mycoides subspecies mycoides (Gladysdale) colonies appeared after 48 hours. Even on primary isolation no difficulty was experienced in isolating and culturing F30. In addition F30 and these reference strains were readily recovered from the tissues of inoculated experimental animals.

Prior to the use of VFG media growth of the F38 strain was difficult. Growth occurred readily in VFG50 when 3.3 ml volumes were incubated in closed bijoux bottles. Titres of $10^7 - 10^9$ cfu/ml could be obtained by daily passage using an inoculum of 1/10th of broth volume. During early passes after primary isolation titres did not exceed $10^7$ cfu/ml, gradually improving as the passage level increased. F38 did not grow satisfactorily in VFG50 medium prepared with rabbit serum in place of goat serum.

F38 colonies became visible after 2 - 3 days incubation at $37^\circ C$ on VFG agar. When plates were incubated in an atmosphere of 5% CO$_2$ and 95% H$_2$ or 10% CO$_2$, 85% N$_2$ and 5% O$_2$ colonies seemed slightly larger than those on plates incubated aerobically. Colonies appeared after 2-3 days incubation at $37^\circ C$ in all atmospheric conditions.
Fermentation Tests

F30, PG3, and N108 lowered the pH of media containing 8 substrates (Table 35) by 1.8 or more and the cultures died within 1 week at 37°C. The pH of the other media fell by 1 or less and the cultures remained alive, with the exception of cultures grown in medium containing sorbitol. The pH of the last medium was lowered by 1.6 - 2.3, depending on the strain tested, PG3 and N108 cultures remaining alive while F30 cultures died on the sixth day. The pH of cultures grown in medium without added carbohydrate fell by 0.7 - 0.75. M. mycoides subspecies mycoides (Gladysdale) fermented these carbohydrates in the same way with the exception that the pH of cultures containing laevulose and sorbitol did not fall my more than 1. Cultures grown in media containing glucose, maltose, mannose, and glycogen died, while the pH of culture grown in medium without added carbohydrate fell by 0.4.

Strains Vom, Smith, and F38 were tested only for their capacity to ferment glucose. Vom and Smith fermented this sugar vigorously and F38 fermented it more slowly, not reducing the pH by more than 1.

Haemolysis

F30, PG3, N108, and M. mycoides subspecies mycoides (Gladysdale) caused clear haemolysis with a light brown to yellow border when grown on solid medium containing defibrinated blood, either horse, goat, sheep, guinea pig, or chicken. Smith, Vom, and F38 were tested only on medium containing defibrinated horse blood, with a similar result. F38 caused clear haemolysis with a slightly green tinge which faded to brown-yellow after further incubation.

Peroxide Production

All strains showed production of peroxide by black discolouration.
Table 35. Fermentation tests

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Fermentation by strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F30</td>
</tr>
<tr>
<td>glucose</td>
<td>+</td>
</tr>
<tr>
<td>Laevulose</td>
<td>+</td>
</tr>
<tr>
<td>mannose</td>
<td>+</td>
</tr>
<tr>
<td>maltose</td>
<td>+</td>
</tr>
<tr>
<td>trehalose</td>
<td>+</td>
</tr>
<tr>
<td>starch</td>
<td>+</td>
</tr>
<tr>
<td>dextrin</td>
<td>+</td>
</tr>
<tr>
<td>glycogen</td>
<td>+</td>
</tr>
<tr>
<td>sorbitol</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: + : pH fell by 1.8 units or more
      † : pH fell by 1 to 1.8 units
      - : pH fell by less than 1 unit

M.G. : *M. mycoides* subspecies *mycoides* (Gladysdale)
of benzidine blood agar within 24 - 48 hours. Under anaerobic conditions no black discolouration and only very slight haemolysis was observed. F38 was not tested under anaerobic conditions.

**Methylene Blue Reduction**

PG₃, Vom, F30, and N108 reduced methylene blue overnight; *M. mycoides* subspecies *mycoides* (Gladysdale) and F38 caused reduction by the third day; Smith did not cause complete reduction. All the cultures reverted to a green-blue colour by the sixth day of incubation.

**Liquefaction of Inspissated Medium**

F30, PG₃, N108, and *M. mycoides* subspecies *mycoides* (Gladysdale) did not liquefy inspissated medium containing pig serum. The other strains were not tested on this medium. The reference strains together with F30 and F38 liquefied inspissated medium containing goat and bovine serum, F38 also liquefied inspissated medium containing horse serum. The caprine organisms, except for F38, liquefied the media more strongly than *M. mycoides* subspecies *mycoides* (Gladysdale). The medium containing inspissated bovine serum was less readily liquefied by the caprine strains, especially F38.

**Growth in Medium without Serum.**

Colonies of F38 were not observed on VFG agar without serum even after prolonged incubation.

F30 formed minute colonies with no papillae on Newing's tryptose agar without serum after 4 days incubation. However this organism formed similar colonies after only 24 hours incubation on VFG agar without serum.
Sensitivity to Digitonin

All the reference strains, F30 and F38 were sensitive to digitonin.

Sensitivity to Antibiotics

No difference in titre was observed in broth cultures of F38, F30, PG₃, N108 or M. mycoides subspecies mycoides (Gladysdale) grown in medium with and without penicillin. Similarly ampicillin did not affect growth of these strains in broth culture (Table 36).

Viability Tests

At 4°C F30, N108, PG₃ and M. mycoides subspecies mycoides (Gladysdale) stored at 4°C maintained a titre of over $10^5$ cfu/ml for 9 weeks. In contrast F38 fell from a titre of over $2.2 \times 10^7$ cfu/ml to less than $10^3$ cfu/ml after 18 days.

At room temperature A drop in titre similar to that observed at 4°C was observed after 10 days when F38 cultures were maintained at room temperature. Room temperature varied from 16 - 24°C.

At -20°C Routinely broth cultures containing high titres of all mycoplasma were stored at -20°C. During this project a large drop in titre was not observed to follow storage at this temperature.

A proportion of the F38 isolates were not viable after 2 to 3 years storage. This was attributed to the use of suboptimal media during the early part of the study. F38 type isolates grown in VFG50 broth and stored at -20°C maintained a high titre.

Lyophilised F30, PG₃, N108, F38 and M. mycoides subspecies mycoides (Gladysdale) cultures were viable after 3 years in the freeze dried state.

Pathogenicity for Embryonated Eggs

All the caprine strains grew in embryonated eggs, killing the embryos in less than 7 days. The LD₅₀ of F30, PG₃ and N108, calculated by the method of Reed and Muench (1938) were 2.2 cfu, 3.7 cfu and 177 cfu respectively.
### Table 36. Plate antibiotic sensitivity tests

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Smith</th>
<th>Vom</th>
<th>PG</th>
<th>N108</th>
<th>F30</th>
<th>M.G.</th>
<th>P38</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Penicillin</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>PS</td>
<td>S</td>
</tr>
<tr>
<td>Sulphonamide</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>R</td>
<td>R</td>
<td>PS</td>
<td>PS</td>
<td>S</td>
<td>PS</td>
<td>S</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Furazolidone</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Neomycin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

**Key:**  
- **R**: resistant, no effect on colony growth  
- **S**: sensitive, complete inhibition of colony growth  
- **PS**: partially sensitive, reduction in rate of colony growth  
- **M.G.**: *M. mycoides* subspecies *mycoides* (Gladysdale)
After F30 had been passaged 50 times in embryonated eggs 1 ml of infected egg fluid containing $2.4 \times 10^9$ cfu was inoculated intramuscularly into the right thigh of each of 3 goats. All 3 goats died within 1 week with symptoms and lesions similar to those observed following inoculation of early passage broth culture by this route. The inoculum was inhibited by growth inhibiting rabbit serum prepared to F30.

**Conclusion**

All the strains fermented glucose, haemolysed horse red blood cells, produced peroxide, reduced methylene blue, and liquefied inspissated liquid medium. F30 but not F38 grew in media without serum and both strains were sensitive to digitonin.

F38 and *M. mycoides* subspecies *mycoides* (Gladysdale) were differentiated from F30 and the caprine reference strains by their slower growth and weaker liquefaction of inspissated liquid media. Both the strains were either sensitive or partially sensitive to penicillin or sulphonamide, using the antibiotic plate sensitivity test, in contrast to F30 and the caprine reference strains.

In contrast to F30, N108, and PG3, *M. mycoides* subspecies *mycoides* (Gladysdale) did not ferment laevulose or sorbitol. The slower growth of F38 even at a high *in vitro* passage and on special medium clearly separated this strain from all the others. In addition at 4°C the titres of F38 cultures fell more rapidly than those of F30, N108, PG3, and *M. mycoides* subspecies *mycoides* (Gladysdale).

All the caprine reference strains grew readily in embryonated eggs causing a high mortality. Serial passage of F30 in embryonated eggs did not attenuate the pathogenicity of the strain to goats following intramuscular inoculation.
MORPHOLOGY

Light Microscopy

Broth cultures examined by dark ground microscopy. Wet preparations of F30, Vom, PG₃, Smith, and N108 broth cultures revealed short, branching filaments, coccal, and point forms. Most of the filaments were short, with a few branching filaments, although some reached 20 μm in length (Plate 48). Occasional star forms were seen with 4 or more branches radiating from a single locus. Filaments with a beaded appearance (Plate 49), as though made up of a chain of point forms, were also observed. During the log phase of growth more filaments were seen in cultures grown in medium containing 0.5 per cent (w/v) glucose than in those grown in medium without glucose. Filaments disappeared in the former cultures within 24 – 48 hours, but remained in the latter for 7 – 10 days. Broth culture of M. mycoides subspecies mycoides (Gladysdale) grew more slowly and developed much longer, more elaborate filaments.

F38 showed filaments similar to those of M. mycoides subspecies mycoides (Gladysdale) except that they were more delicate in appearance, possibly due to thinner elements. These structures bore the closest resemblance to the filaments seen in pleural fluid and exudate from the lungs of field cases. In cultures grown in medium containing 0.5 per cent (w/v) glucose filaments were observed for several days, being much more numerous in high titre cultures.

Colonies. Colonies of F30, Vom, PG₃, Smith, and N108 were indistinguishable. After 16 – 24 hours incubation very small
Colonies were visible, reaching 0.5 - 1 mm in diameter by 48 hours and slowly enlarging to 3 - 5 mm by the eighth day. Young colonies had smooth surfaces and a typical 'fried egg' appearance. With age their outline became irregular, their surface roughened and wrinkled, and by 4 - 5 days multiple papillae developed, increasing in number to cover the whole colony surface (Plate 50).

Colonies of *M. mycoides* subspecies *mycoides* (Gladysdale) were not visible until after 48 - 72 hours incubation and did not grow so large, although the same changes occurred with age.

F38 colonies appeared after 2 days incubation on VFG solid medium prepared with agar Noble. The colonies were only just visible at x30 at this time, slowly enlarging to reach 0.2 mm in diameter after 4 - 5 days incubation. Apart from their size the colonies resembled those of the other strains except that although the colony surface became roughened and wrinkled after 5 days incubation multiple papillae were not observed.

**Colony sections of F38.** Thin sections stained with toluidine blue and examined by light microscopy showed that the central and multiple papillae corresponded to down-growths into the agar (Plates 51 and 52). In many there was a line separating that part of the colony in the solid medium from the surface layer. Cells within the agar stained more darkly than those above the separating line, the most deeply staining cells being at the colony-to-agar interface.

**Electron Microscopy of F38**

**Colonies.** Cells in colony sections examined by electron microscopy were characterized by their site within the colony and by the distribution of their ribosomes: either even; peripheral; or
irregular. Those with evenly distributed ribosomes ranged from small, regular, densely staining bodies, 75 - 250 nm in diameter, to larger, paler, irregular cells, 250 - 1200 nm in diameter. Only a few small bodies were present throughout the sections, their number appearing to increase slightly as the colony aged. In some cells, especially the dense, small bodies, it was not possible to distinguish ribosomes. The majority of cells with evenly distributed ribosomes were localized in the centre of colony sections (Plate 53). These cells were usually 500 - 1200 nm in diameter, and a fine, fibrillar, network was sometimes visible in the cytoplasm. A third type of cell with evenly distributed ribosomes was regular, oval to circular in shape, 250 - 1000 nm in diameter, and found only in 'air phases'. One or two 'ring forms' were observed among the cells at the colony-to-agar interface.

Cells with a peripheral arrangement of ribosomes were found mainly at the colony-to-agar interface, although a few were seen at the colony surface (Plate 54). These cells were irregular in shape and ranged from 250 - 1000 nm in diameter. Frequently faint staining, amorphous material was seen in the otherwise clear central zone, where in many cells delicate, interwoven fibrils were also present. Similar cells, but with irregularly distributed ribosomes, were seen in small numbers throughout the colonies, especially at the colony-to-agar interface.

Elongated cells up to 2 μm in length and 100 - 150 nm in width were found with evenly, peripherally, and irregularly arranged ribosomes. They were present at the colony-to-agar interface, and sometimes in the centre of colonies, but never in large numbers. On occasion, 2 or more spherical parts of a cell were joined by an
In one observed instance the ribosomes were clearly localized in the spherical regions.

In all types of cell the ribosomes were often superimposed on small areas of diffuse, homogeneous material, which stained lighter or darker than the ribosomes. This material was not confined to specific sites, and sometimes it was not overlaid by ribosomes.

Sections of 48-hour colonies showed very few apparently dead cells in the centre, although considerable numbers were present at the colony-to-agar interface. Dead cells appeared as empty sacs or as vesicles enclosing irregularly distributed, amorphous, electron dense material. At the colony-to-agar interface small numbers of budding cells were usually observed.

With age the proportion of dead cells in all parts of colonies increased. In the centre of 4- and 8-day-old colonies, foci of live cells were easily distinguishable as discrete, more densely staining areas. The foci were often aligned with subsidiary down growth of the colony into the agar. In these colonies, although most of the cells were dead, cells apparently budding were present at the colony-to-agar interface.

Cells containing 1 or more membrane-limited vacuoles were occasionally seen, especially in 8-day-old colonies. These cells were often irregular, 500 - 1000nm in diameter, with a rather amorphous internal structure. One cell contained a small body, approximately 130 nm in diameter, within a membrane limited vacuole.

Cells called 'ring forms' were smaller, 250 - 650 nm in diameter, circular to oval, and regular in shape, the membrane limited 'vacuole' occupying more than half of the cell section.
The cytoplasm of ring forms was generally filled with evenly distributed, sharply defined ribosomes.

In a number of sections the structure of the cell membrane was seen to consist of 2 electron dense layers separated by an electron lucent layer. The layers were approximately the same width, the whole membrane being 7 – 8 nm thick.

Cells apparently dividing occurred at the colony-to-agar interface, where up to 5 per cent of the cells were sometimes involved. Occasionally, such cells were seen in the deeper parts of the surface layer. Generally parent cells were 250 – 1000 nm in diameter, usually with peripheral or irregularly distributed ribosomes. Prior to division electron dense material and ribosomes concentrated in a discrete clump below the cell membrane, forming a bud (Plate 55). Daughter cells would be small, electron dense, and closely packed with ribosomes. Sometimes a parent cell was seen forming several buds. Infrequently cells evenly filled with ribosomes seemed to be dividing into equal portions.

In 10 colonies some parts of the surface showed additional oval cells, evenly filled with ribosomes, and varying from 250 – 1000 nm in diameter. The smaller cells with densely packed ribosomes stained deeply. These cells greatly altered the appearance of the surface in colony sections, and, where present, the area was called an 'air phase' (Plate 56). An air phase was not observed to cover the entire colony surface in any section. In some sections the air phase was separated from the centre cells by an electron dense or electron lucent band. This surface formation was seen in all three ages of colony, grown in medium with and without glucose, and fixed by either method.
In three 4-day-old colonies grown on medium containing glucose, a small number of cells contained a remarkable, striated intracellular structure (Plates 57 and 58). A total of 40 affected cells were seen. In the few cells where the plane of section permitted accurate measurement, the structures were seen to consist of electron dense and electron opaque bars with a periodicity of 11 - 12 nm. Where the ends of the structure were visible they were capped with electron dense, homogeneous material, and lay just below the cell membrane. Affected cells were either moderately irregular, 300 - 800 nm in diameter, or, more often, elongated up to 2 \( \mu m \). Occasionally the striated structure joined 2 or more spherical portions of an elongated cell.

The distribution of cell types within colony sections was the same with either method of fixation. However cells fixed with glutaraldehyde were more regular in shape, had less clearly defined ribosomes, and showed a greater variation in density of staining. With both fixatives occasional ribosome-filled cells had a narrow, electron-lucent zone just below the cell membrane. The striated intracellular structures were seen solely in those fixed by glutaraldehyde and postfixed with osmium tetroxide.

Broth cultures. In sections of centrifuged pellets from the 24-hour culture grown in medium without glucose, there were few dead organisms. The cells varied in diameter from 75 - 1000 nm, being regular in shape with evenly distributed ribosomes. The predominant cells were 500 - 700 nm in diameter, and a few of these had irregularly distributed ribosomes. In some of the less densely staining, larger cells, a fine, fibrillar network was present in the centre. Approximately 30 per cent of the cells were ring forms and a similar number were elongated up to 2 \( \mu m \).
The elongated cells were often sickel or dumb-bell shaped, and sometimes branched. Only a few small bodies (75 - 250 nm) were present, and few apparently dividing cells were observed.

In sections of pellets from older cultures there was an increase in the number of cells with peripherally and irregularly distributed ribosomes, and fewer ring forms. The number of apparently viable cells progressively decreased, and in the pellet from the 8-day culture grown in medium without glucose most of the cells appeared dead. The cells observed in pellet sections from the 24-hour culture grown in medium containing glucose were similar to those in the pellet sections from 8-day culture in medium without glucose.

Cells fixed before centrifugation showed slightly better defined internal structure compared with those fixed after centrifugation. However cells in sections of pellets were generally not so sharply defined and showed less detail than those seen in sections of colonies.

**Negative stained preparations.** Negatively stained preparations showed irregular to oval shaped bodies connected, and in some cases covered, by electron dense, homogeneous material. Their sizes and shapes corresponded well with those recorded for similar bodies seen in colony and pellet sections.

**Conclusion**

All the strains formed typical mycoplasma colonies on solid medium and filaments in broth culture. *M. mycoides* subspecies *mycoides* (Gladysdale) and F38 were distinguishable from the other strains by their slower growth and more elaborate filaments. F38 filaments were more delicate than those of *M. mycoides* subspecies *mycoides* (Gladysdale).
Examination of colony sections of F30 by light and electron microscopy showed the central and multiple papillae to be downgrowths into the solid medium and revealed a line separating surface and deeper colony growth.

Cells in colony sections studied by electron microscope were characterized by their site within the colony and by their distribution of ribosomes. A remarkable striated intracellular structure was observed in a small proportion of cell sections. Cells in negatively stained preparations were obscured by electron dense homogeneous material.
Morphology of F30

Plate 48. Branching filament, phase contrast x 1500
Plate 49. Beaded filament, phase contrast x 1500
Plate 50. 8 day colony, x 10.7
Plate 51. Cross section of an 8 day colony, x 11.4
Plate 52. Cross section of an 8 day colony, x 122
Plate 53. Colony centre cells, 40 x 10^3
Plate 54. Cells with peripheral ribosomes showing DNA-like fibrils in the centre, 51 x 10^3
Plate 55. Budding cell, 31.2 x 10^3
Plate 56. Air phase above colony centre cells, 17.5 x 10^3
Plate 57. Striated intracellular structure, 68 x 10^3
Plate 58. Striated intracellular structure, 65 x 10^3
DISCUSSION

The field condition studied closely resembles descriptions by previous authors (Longley, 1940, 1941). Two of the symptoms in the disease were described in the microscopical lesions mentioned in the study of different stages of the disease attributed to the study of different stages of the disease.

Longley (1940) described the condition as acute and relatively transient rise in temperature to about 41 degrees C. In contrast, Bosc (1946) found initially a rise of temperature to approximately 105 degrees F. In the acute phase, death occurred in three to four days. In the subacute phase, death occurred in four to seven days. In the chronic phase, death occurred in two to four weeks.

While some did not recover, others did not. Deaths were observed in small experimental areas of the experimental susceptible population.
The aim was to find an aetiological agent for contagious caprine pleuropneumonia in Kenya, preferably an agent which would initiate an experimentally contagious pleuropneumonia. This was achieved in that isolates of the F38 type were shown to fulfil Koch's postulates. In addition the pleuropneumonia they initiated was contagious spreading not only from inoculated donor goats to susceptibles but from contagiously affected goats to susceptibles.

The main aspect became the study of field cases of CCPP sent to the Veterinary Research Laboratories, Kabete, by field officers in different parts of the country. I have been primarily concerned with the acute epidemic syndrome because not only is this the most economically important form of the disease but acute cases would seem more likely to harbour the aetiological agent.

The field condition studied closely resembled that described by previous authors (Longley, 1940; 1951; Mettam, 1929; Shirlaw, 1949). Some of the variation in the description of the clinical syndrome and macroscopic lesions mentioned in the review can be attributed to the study of different stages of the disease. Longley (1940) described the condition as afebrile although a transient rise in temperature to about 40°C was not rare; in contrast Bawa (1946) found invariably a rise of temperature to approximately 41°C. I have observed, in the same outbreak, both febrile cases which were acute and afebrile cases with a more prolonged course. In this study some cases showed nasal discharge while others did not. However nasal discharge was not a feature of the experimental contagious disease.

I would emphasize that although enlargement of interlobular septae was observed in small peripheral areas of larger hepatized
lesions it was not a prominent feature and consequently there was
never any marked similarity in the macroscopic appearance of the
acute lung lesions of CCPP and those of CBPP. In this respect my
observations differed radically from those of Cottew and Leach (1969)
and Shirlaw (1949). In addition the mosaic of colours seen in
hepatized lesions was more striking than that described by previous
authors (Longley, 1940; Shirlaw, 1949). While the pericardium was
frequently thickened with fibrin in only one case was the epicardium
affected as well. In contrast to the observations of Christodoulu
and Talartzis (1957) arthritis and lameness were not features of the
field cases of CCPP which I studied.

During this work mycoplasma identified as F38 by growth
inhibition test were isolated from 30 acute outbreaks. Throughout
the study no mycoplasma similar to M. mycoides subspecies capri (PG3,
Smith, and N108) or M. mycoides subspecies mycoides (M. mycoides
subspecies mycoides strain (Gladysdale) and Vom strain) were isolated
from acute cases of CCPP.

From 3 cases of chronic caprine pleuropneumonia derived from
2 separate goat herds organisms of the F30 type were isolated. Both
goat herds had suffered an outbreak of CCPP several months previously.
From one of these herds during the acute phase of the disease 3 cases
were examined culturally and F38 only was isolated. Since F30 grew
very luxuriantly even on primary isolation it is unlikely that this
agent was present in the 3 acute cases.

It is widely recognized that reference aetiological agents of
CCPP are difficult to isolate (Cottew and Leach, 1969), therefore
the search for similar agents was continued. However in view of
the tenuous data relating any of the reference agents to the natural
disease F38 was chosen for more detailed study. At the same time
the similarity of F30 to reference agents of CCPP invited further
investigation. Since F30 grew readily \textit{in vitro} the study of this
agent progressed more rapidly than the investigation of F38.

In view of the urgency to establish the main aetiological agent
of CCPP in Kenya the significance of F38 and F30 in the field disease
and their experimental pathogenicity to goats was given priority
over their classification within the \textit{Mycoplasma}tales. For the same
reason mycoplasma isolated only infrequently and not affected by
growth inhibiting sera to reference agents of the \textit{M. mycoides}
species, F38, or F30 have yet to be studied further. Similarly
no one bacterial species was consistently isolated. The search for
viruses was curtailed when it had become apparent that F38 type
mycoplasma were present in a high proportion of acute field cases
of CCPP. The aim was not only to isolate a virus but to investigate
whether tissue cultures would be suitable for isolation of F38 type
mycoplasma. The tissue cultures used in this study were not
satisfactory for the isolation of F38.

However it is widely recognized that mycoplasma, bacteria, and
viruses can complicate pneumonias primarily initiated by other agents
or stimuli. Indeed such agents may play an important role in the
natural syndrome. Beaton (1931) suggested that the mycoplasma he
isolated from CCPP could be a \textit{'bacille de sortie'} although later
Longley (1951) considered Beaton had isolated the aetiological
agent of CCPP.
EXPERIMENTAL PATHOGENICITY OF F30
AND REFERENCE AGENTS OF THE M. MYCOIDES SPECIES

F30 was pathogenic, causing pleuropneumonia, connective tissue lesions, and muscle lesions in goats and sheep following experimental inoculation. In these respects the organism closely resembled agents of CCPP described by Longley (1940, 1951) and Shirlaw (1949).

In the present study the subcutaneous lesions in the thigh and behind the elbows which occurred following endobronchial inoculation of 1 of the donor goats were unexpected. The contact goats took particular exception to this donor animal and it may be these lesions containing the organism marked the site of tissue damage inflicted during fighting. The isolation of F30 from foetal fluid and tissues of another of the donor goats is not without precedent. M. mycoides subspecies capri has been recorded to infect the foetuses of experimentally inoculated sheep (Longley, 1951) and goats (Yedloutschnig et al., 1971); similarly M. mycoides subspecies mycoides may cross the placenta in cattle (Stone, Masiga, and Read, 1969) and in sheep (Beller and Tahssin-Bey, 1926). F30 did not cause disease transmissible by contact although in-contact goats developed complement fixation test antibodies to this organism.

The inoculation of cattle with agents of CCPP or crude exudates from field cases of this disease has given variable results. Barber and Yedloutschnig (1970) and Provost (1966) report fatal infection while Lindley and Abdulla (1969), Longley (1951), Mettam (1929) and Shirlaw (1949) record no infection.

Before classifying F30 culturally and serologically in relation to reference strains of the M. mycoides species it was
necessary to assess their experimental pathogenicity to goats where this was in doubt, particularly because there is little other data relating such agents to CCPP as it occurs in the field. Strain 'Smith' was not included in this aspect of the work because its pathogenicity to goats following inoculation has been established recently (Cottew et al., 1969) and confirmed by Goni and Onoviran (1972).

PG₃, the world reference strain of \textit{M. mycoides} subspecies \textit{capri}, did not cause clinical disease, specific lesions, or complement-fixing antibodies in inoculated goats or goats in contact. Cottew et al. (1969) found their strain of PG₃ to be avirulent, but their agent has been shown to be PP goat (Lemcke, 1974). At the time of isolation PG₃ caused pleuropneumonia in 1 of two goats (Cottew and Leach, 1969) but subsequently El Nasri (1967) found this agent to be avirulent. Although the original organism may have become attenuated or even lost in the intervening years there was very little evidence that this organism had an aetiological role in CCPP as it occurs in the field.

N108 also failed to cause clinical pleuropneumonia or specific lung lesions. However in the intratracheal endobronchial experiment 11 out of 12 goats, including 3 in-contacts, developed complement-fixing antibodies ranging from 1/16 to 1/256 in titre. In the intramuscular experiment, 3 goats showed transient clinical symptoms and all developed complement-fixing antibodies. This agent therefore was more pathogenic than PG₃ and its ability to give rise to antibodies in goats kept in contact with others which had been inoculated was of particular interest. It is regrettable that the history of this agent is so vague (Cottew and Leach, 1969).
The Vom strain was highly pathogenic to goats by the intratracheal endobronchial route and intramuscular route of inoculation, killing 13 out of 14 inoculated goats with an experimental disease indistinguishable from that caused by F30; similarly all affected tissues cultured at death or after euthanasia yielded the organism. Only 1 of the 4 in-contact goats developed a complement fixation antibody titre of 1/16. Affected goats developed a marked leucopenia, reflecting a sharp decline in the numbers of lymphocytes, and neutrophils. Yedloutschnig et al. (1971) also observed marked leucopenia in goats affected following inoculation of strain 208.

Of the 3 caprine mycoides reference strains tested, Vom was undoubtedly the most pathogenic. It is of interest that Cottew et al. (1969) found their Vom strain to be avirulent to goats, while our results and those of Barber and Yedloutschnig (1970) indicated this agent was highly virulent to goats.

Serological and cultural comparison of these agents revealed a close relation between M. mycoides subspecies mycoides (Gladysdale), F30, and Vom. For this reason the pathogenicity of M. mycoides subspecies mycoides (Gladysdale) for goats was investigated.

M. mycoides subspecies mycoides (Gladysdale) caused transient clinical reactions in 5 out of 14 inoculated goats. Only 2 goats, inoculated intramuscularly, developed a complement-fixing antibody response. In the literature, inoculation of goats with M. mycoides subspecies mycoides showed very variable results (Abdulla and Lindley, 1967; Beller and Tahsin-Bey, 1926; Dick, 1937; Lindley and Abdulla, 1969; Turner et al., 1935). However overall M. mycoides subspecies mycoides of bovine origin is not considered to be as pathogenic for goats as those agents of CCPP so closely
related to the former as to be termed \( M. \) mycoides subspecies mycoides of caprine origin (Al-Aubaidi et al., 1972).

The occurrence of arthritis in goats inoculated with F30 was not unexpected since both Longley (1951) and Christodoulu and Talartzis (1957) recorded arthritis as a feature in natural cases of CCPP. The distribution of reisolates in goats inoculated with F30 and Vom suggested mycoplasmaemia as a feature of the experimental disease. Shirlaw (1949) made a similar observation.

That F30, Vom, and N108 initiated complement-fixing antibodies in contact goats indicated that these agents could infect contact animals although under the conditions of the experiments they failed to cause disease. The experimental animals were in excellent condition and maintained optimally, and it may be these agents can cause disease when goats are subjected to environmental stress factors which occur in the field, for example, poor grazing, inclement weather, transportation. While stress factors may be critical to the manifestation of infection with these agents it is difficult to credit that the agent of so virulent a condition as natural CCPP, as it occurs in Kenya, could be so dependent.

Several authors (Longley, 1940, 1951; Mattam, 1929; Shirlaw, 1949) demonstrated in-contact transmission of the natural disease and the disease induced with exudates and extracts of lesions from field cases. Apart from Yedloutschnig et al. (1971) there was no record of in-contact transmission of disease induced by cultured organisms. It is noteworthy that while the last authors achieved this result with organisms classified as \( M. \) mycoides subspecies capri these were not isolated from the classical disease and
belonged to Group 8 of Al-Aubaidi et al., (1972) which included organisms so closely related to the aetiological agent of CBPP as to be termed M. mycoides subspecies mycoides of caprine origin. Although F30 and the Vom strain belonged to this group similar agents were not isolated from acute cases of CCPP during this study.
EXPERIMENTAL PATHOGENICITY OF F38

The preliminary experiment with F38 was totally negative. The inoculum in this experiment comprised a pool of cultures which had been passaged from 30 to 77 times in artificial media. At this stage of the investigation Newing's tryptose media were being used for the growth of strain F38. This medium did not support optimal growth of strain F38. The F38 and G69 inocula used in subsequent trials were grown solely in VFG media and only passaged 10 - 16 times in vitro prior to inoculation. The negative results were therefore attributed to the inoculum used.

In the second experiment only 3 out of 8 of the inoculated goats became affected and only 2 of these died from the specific disease, while 3 out of 4 contact goats developed specific lesions. This could indicate the agent is more virulent following contact transmission. Alternatively, with reference to results of later contact experiments with this agent, it is tempting to speculate that the inoculum may have vaccinated the other 5 inoculated goats which remained unaffected.

In the third experiment, in view of the low virulence of F38 following inoculation, it was decided to employ a more recent, similar isolate from an acute outbreak in Garissa. This isolate, G69, was specifically inhibited by antiserum prepared to F38. In an attempt to promote pneumonia in inoculated goats an intravenous technique involving prior injection of chloroform followed by a pure culture was employed (Longley, 1951).

The very prominent and enlarged grey interlobular septae observed in the lungs of 2 goats injected intravenously with
chloroform followed by culture were not present in the lungs of
contact cases. It is probable these lesions were attributable to
the method of inoculation. This technique may not have promoted
pleuropneumonia in the inoculated goats significantly, since the
second 2 inoculated goats to be affected showed symptoms 39 and 42
days p.i.. So long an apparent incubation period may well indicate
the animals were in fact infected or reinfected by contact later in
the experiment. The first inoculated goats showed symptoms 14 days
later. It is of note that in the previous F38 experiment the
inoculated animals showed an incubation period of 8 - 15 days.
Overall the minimum incubation period for the contact experimental
disease ranged from 11 to 21 days. Although there is at present
insufficient data to establish an incubation period it would seem
to be longer than those observed in pathogenicity experiments with
F30, Vom, and N108, where the disease arose from inoculation.
In the contact challenge experiment with F38 and G69 the agent
was reisolated from the lung lesion and mediastinal lymph nodes of
one goat as long as 33 days after the febrile phase. At this time
the lesions were fibrous and necrotic, a stage generally considered
not infective (Longley, 1940, 1951). Previous inoculation of 6 goats
with high passage F38 culture appeared to afford a measure of
protection against the contagious disease initiated by G69, although
two of the goats were susceptible. One of the susceptible goats
showed a biphasic febrile reaction at a 26 day interval suggesting
it was unable to acquire immunity. In 2 of the 4 protected goats the
agent was reisolated from the retropharyngeal lymph nodes at slaughter
62 days post exposure. These isolates may have been F38 from the
original inoculation or G69 from contagious infection.
The final intratracheal endobronchial experiment with G69 confirmed the previous experiments. However further information was obtained on the serological response of inoculated and in-contact cases and sera from these animals were used to confirm the relationship of F38 to the M. mycoides species by the agar gel diffusion test.

There is a clear need for further pathogenicity experiments to be undertaken with particular reference to different routes of inoculation and the pathogenesis of the condition. Nevertheless isolates of the F38 type were able to cause caprine pleuropneumonia experimentally and moreover this condition was very contagious to healthy goats. Indeed F38 would seem to be not only a pathogenic mycoplasma commonly isolated from acute field cases of CCPP but also the only mycoplasma isolated from this disease which has been reported to initiate experimental caprine pleuropneumonia which was readily contagious. The macroscopic lesions caused by F38 experimentally resembled those of the natural disease in Kenya more closely than those initiated experimentally by F30 and Vom strains, (Kaliner and MacOwan, 1976).

The experiments conducted during this work were of particular significance since all the goats were derived either from the laboratory breeding herd at the Kabete Veterinary Research Laboratories or from farms with herds known to be free from CCPP. From the laboratory breeding herd, established in 1970, only progeny were used experimentally. At no time has CCPP been diagnosed in this herd and up to the present these animals have been free of complement-fixing antibodies to F30 and F38.

The goats employed in P03, M108, Vom, M. mycoides subspecies
mycoides (Gladysdale) experiments, and in the last experiments with G69 were all free from complement-fixing antibody to the agent inoculated. In view of the cross reactions between F30, F38, and reference agents revealed by complement fixation test and agar gel diffusion test with field goat sera and sera from goats experimentally infected with G69 it is likely that the goats were free of antibody to the M. mycoides species as a whole. In the earlier experiments with F30 and F38 the goats were not screened by complement fixation test since the test had not been developed. However these animals derived from the same sources as those employed subsequently.

All control goats in the F30 intratracheal endobronchial and second F38 experiments were killed and found to be free of pleuropneumonia. The goats inoculated intratracheal endobronchially with strains PG3, N108, and M. mycoides subspecies mycoides (Gladysdale) served as further controls. The experimental animals inoculated with G69 by the intratracheal endobronchial route were selected by random tables and paired. In this experiment goats were screened culturally for viruses as well as bacteria. In contrast goats used in earlier experiments were only culturally screened for mycoplasma and bacteria.

The sheep and cattle used in pathogenicity experiments with F30 were the progeny of established herds at the Veterinary Research Laboratories, Kabete. In addition the animals were found to possess no complement-fixing antibodies to F30 prior to experiment.

Each experimentally infected group was controlled by the maintenance of an equal number of control animals from the same source in different premises but otherwise treated in the same way. When the animals in the infected group were inoculated their controls received
an equal volume of uninoculated mycoplasma medium by the same route. The confirming of reisolated mycoplasma to be the same as those inoculated by growth inhibition test provided a further specificity control for experiments.
SEROLOGICAL RESULTS

Serological Comparison of Strains Using Reagents from Field and Experimental Cases

Antigen of the *M. mycoides* species was demonstrated by agar gel double diffusion test in lung lesions of field cases of CCPP. In 13 out of 39 cases this antigen was common to *M. mycoides* subspecies *capri*, PG₃, N108, and Smith, *M. mycoides* subspecies *mycoides* (Gladysdale) and group 8 Vom strain (Al-Aubaidi et al., 1972), as well as to F30. Similarly 5 out of 26 sera from 3 out of 9 field outbreaks of CCPP reacted with antigen to all these strains and to F38. The complement fixation tests with these sera showed the majority possessed antibody to both F30 and F38 antigens.

Cross-reactions between CCPP agents shown with sera from field cases are not without precedent. El Nasri (1967) found such sera reacted with both GPS and OSB42 by slide agglutination test. However it was not unexpected that my antigens autoagglutinated since others have encountered this problem (Lemcke, 1964).

To some extent the above findings were supported by the results of the agar gel double diffusion test using lung lesion from F38 and G69 experimental cases. Lesion material from 5 cases reacted with the hyperimmune sera prepared to F30 and the reference strains. Sera from two experimental cases showed a clear antigenic relationship between F38, PG₃, Vom, F30 and *M. mycoides* subspecies *mycoides*. By complement fixation test sera from 15 experimental cases initiated by F38 and G69 isolates reacted with both F30 and F38 antigens. Further work with high titre clinical sera is indicated.

The non-specificity of the agar gel double diffusion test and complement fixation test within the *M. mycoides* species may not
detract from their diagnostic value. More than one member of the
M. mycoides species has not yet been isolated from an individual
case of CCPP. The results using lung lesion material from F38 and
G69 experimental cases suggested the presence of a common antigen in
field cases rather than multiple infection with mycoplasma of the
M. mycoides species. It may be that in only a limited number of
cases was there sufficient F38 antigen in the lesion to cross react
in these tests. To assess the sensitivity of the agar gel double
diffusion test it would be necessary to prepare a hyperimmune serum
to F38 which was free of antibody to goat tissues. The hyperimmune
serum which I prepared in rabbits with washed F38 grown in medium
made from goat tissues regrettably reacted with goat tissues.
Before undertaking the preparation of this goat antiserum inoculation
of F38 into goats by different routes must be investigated.

Lesion material from experimental cases initiated by F30 also
reacted with hyperimmune rabbit sera to PG3, N108, Smith, Vom, M.
mycoides subspecies mycoides (Gladysdale) and F30 again confirming
these mycoplasma to be very closely related. Similarly complement
fixation tests with sera from one of the chronic outbreaks from which
F30 was isolated reacted both with F30 and F38 antigens. Since F38
and F30 were isolated at different times from another CCPP outbreak
these results may reflect dual infection. However it must be
recalled F30 was isolated during the convalescent or chronic phase
of the outbreak and this was reflected by the complement fixation
test results in which a rising titre was observed to F30 only.
However more work following the course of CCPP in different herds
culturally and serologically would be necessary to establish if F30
has any particular significance to the natural disease.
Serological Comparison of Strains Using Hyperimmune Rabbit Sera and Concentrated Antigens

The findings of the agar gel double diffusion test, complement fixation test, and growth precipitation test supported the relationship found between F38, F30, and reference mycoplasma using reagents from field and experimental cases.

The complement fixation test enabled *M. mycoides* subspecies *mycoides* (Gladysdale) to be distinguished from the caprine strains. This result was confirmed using sera from 2 cattle with contagious bovine pleuropneumonia. More work would be required to establish the fact that such clinical sera do not react significantly with other members of the *M. mycoides* species. While the growth precipitation test supported the agar gel and complement fixation test findings F38 culture did not react with hyperimmune sera prepared to F30 or to the reference mycoplasma of the *M. mycoides* species. This may have been a reflection of poor multiplication of this agent under the test conditions rather than the specificity of this test, especially as F38 hyperimmune serum reacted with F30 and all the reference mycoplasma.

When large immunizing doses of mycoplasma are used together with adjuvant to prepare hyperimmune sera there may be an increase in relatively non specific antibodies and such sera may show more cross reactions than those prepared with less antigen and without adjuvant (Lemke, 1973). On this basis there may be objection to my agar gel diffusion and complement fixation test results carried out with hyperimmune rabbit sera. However, the fact that field goat sera from herds affected with CCPP reacted with several members of the *M. mycoides* species by agar gel diffusion test and
also the reaction of such sera with both F30 and F38 by complement fixation test indicated that these organisms were related. While even the field sera might be suspect on the basis of multiple infection with members of the \emph{M. mycoides} species, this objection did not apply to the sera of experimental goats affected by CQPP in the F38 and G69 experiments. Agar gel diffusion tests using sera from experimentally infected goats confirmed the relationship of F38 and F30 to other members of the \emph{mycoides} species. Therefore I consider that my agar gel diffusion and complement fixation tests using hyperimmune rabbit sera and goat sera against lesion material or concentrated antigen suggested both F30 and F38 belonged to the \emph{M. mycoides} species. The growth precipitation test also supported this view.

The 5 reference strains, F30 and F38 were satisfactorily differentiated into 3 groups by growth and metabolic inhibition tests, F30 being similar to the Vom strain and \emph{M. mycoides} subspecies \emph{mycoides} (Gladysdale). The tests grouped PG3, N108 and Smith strains together but showed F38 was not related to any of the other strains. The relationship of F30 and F38 to the \emph{M. mycoides} species requires confirmation using the incident light immunofluorescent technique of Tessler (1973b). Further critical classification of these strains may be obtained from mouse cross-protection tests (Arisoy et al., 1969; Smith, 1965, 1967, 1969). This test could perhaps also provide information relevant to vaccine trials in goats using contagious challenge initiated by an F38 type isolate.

It is of note that the Mycoplasma Reference Laboratory, Colindale, and the International Collaborative Centre for Animal Mycoplasmas, University of Aarhus have arrived at a similar
classification of F30 (Appendix). F38 has yet to be classified.

Complete characterisation of F38 is now in process and if it proves ultimately to represent a new serotype a name will be proposed.
CULTURAL RESULTS

Overall, the results of cultural comparison emphasized the similarity of F30, PG, N108, Smith, and Vom. M. mycoides subspecies mycoides (Gladysdale) was shown to differ from this group since it grew more slowly and did not ferment laevulose so well. F38 was readily differentiated from all the others by the difficulty of growing it; even in VP50 broth it grew more slowly. In addition this organism was characterized by only slight fermentation of glucose. Some differences were observed between strains in the rate of reduction of methylene blue and liquefaction of inspissated serum. PG, N108, F30, and M. mycoides subspecies mycoides (Gladysdale) strains all showed similar haemolysis regardless of the species red blood cells tested. This contrasted with the findings of other authors (Cottew et al., 1969). It may be that haemolytic changes were very dependent on the medium and conditions of growth (Cole et al., 1968).

The antibiotic disc sensitivity test was undertaken at a time when growth of F38 was proving difficult and it was considered that routine use of penicillin in the isolation media may have inhibited growth of this organism. The result using the disc sensitivity test which showed F38 to be sensitive to penicillin was not confirmed when the growth of this organism was compared in broth cultures with and without penicillin in the medium. However the disc sensitivity test showed differences between the strains of mycoplasma tested, particularly between F38 and the others. At this time it was also found that ampicillin did not affect the growth of F38, F30, or the reference mycoplasma in broth culture.
For this reason ampicillin was used in tissue cultures of CCPP lesions since an antibiotic of wider spectrum than penicillin was necessary to control bacterial contamination. It is appreciated that the results of disc antibiotic sensitivity tests probably have little application to therapy.

All the caprine mycoplasma studied grew readily, rapidly proving fatal to chicken egg embryos. The LD$_{50}$ of F30, PG$_3$, and N108 did not reflect their degree of pathogenicity to goats. In addition the passage of F30 in embryonated chicken eggs up to the fiftieth pass did not noticeably attenuate the organism for goats.
MORPHOLOGY

The morphology of F30, Vom, PG₃, Smith, and N108 observed in phase contrast was similar and contrasted with the longer, more elaborate elements observed in cultures of *M. mycoides* subspecies *mycoides* (Gladysdale) and F38. The filaments of F38 compared with those of *M. mycoides* subspecies *mycoides* (Gladysdale) were finer and remained plentiful in cultures for a longer period. A similar division of these strains was possible from observations on the rate of appearance and size of colonies.

Examination of colony sections of F30 showed the central and multiple papillae to be down growths into the solid medium. The line separating the surface layer of colonies from the deeper growth may have indicated the former arose by the spreading of cells from the initial subsurface growth at 1 or more discrete points (Razin and Oliver, 1961). This line may be the same as that observed below air phases in sections studied by electron microscope. The electron density and regular shape of air-phase cells could be associated with lower moisture content in their environment at the colony surface. Where an air phase was absent, the surface layer of the colony may have become detached during preparation of the section.

The intracellular striated structure is indistinguishable from the rho forms described by Rodwell et al. (1972, 1973); and Rodwell (1974). Unfortunately the urgent need to establish an aetiological agent for CCPP in Kenya limited the study of this structure observed by Professor Krauss, Department of Pathology, Veterinary Faculty, University of Nairobi, and me in 1971. However I have conducted two experiments in which F30 was intramuscularly inoculated into goats and fixed and embedded lesion material sent to Professor
Krauss, now at Giessen University, West Germany, for electron microscope study. (MacOwan, Kaliner and Krauss, 1975 appendix).

It is probable that large numbers of the ring forms and sickle or dumb-bell shaped elongated cells seen in sections of pellets from broth culture may have been due to the use of hypertonic fixative (Lemcke, 1972). However similar distorted or abnormal shapes were not observed in colony sections. It may be that this effect is less critical when organisms are growing on agar.

The homogeneous, electron dense material covering the cell membrane of organisms in negatively stained preparations has been observed on various mycoplasma species and described as a medium precipitate, organism secretion, or preparative artefact (Black et al., 1972; Domermuth et al., 1964). Only a small amount of this material was present in colony and pellet sections, indicating it derived at least in part from the method of preparation.
CONCLUSION

Two strains of mycoplasma were isolated from pneumonic goats in Kenya, F30 from chronic cases and F38 from acute cases of CCPP. These two strains were compared with strains of M. mycoides subspecies mycoides, Al-Aubaidis group 8, and M. mycoides subspecies capri.

F30 was highly pathogenic to goats following inoculation and this strain belonged to Al-Aubaidi's Group 8. F38 was pathogenic to goats by inoculation and the disease induced was similar to CCPP, spreading readily to in contact goats. The classification of this strain is in process.
APPENDIX
Copies of the reports from Mycoplasma Reference Centres are included, figures 4, 5, 6, and 7.

Their results seem to place F30 as *M. mycoides* subspecies *mycoides*, a member of Al-Aubaidi's Group 8 (Al-Aubaidi et al. 1972). Classification of strain F38 is in process and if it proves ultimately to represent a new species or serotype a name will be proposed.
REPORT for M. 2055/75 received 13.6.75 F.D. ampoule

Tests on this strain gave the following results:

Biological

- Growth on 20% horse serum (+ yeast extract) media: Profuse (aerobically)
- Growth on 2.5% horse serum agar (no yeast extract):
  - Glucose fermentation: positive
  - Arginine hydrolysis: negative
  - Egg yolk agar: no "film & spots"
  - Tetrasodium agar (aerobic): strongly positive
  - Liquefaction of serum (inspissated broth + serum slopes): positive
- Growth on serum-free agar: growing at the 7th sub-
- Sensitivity to sodium polyethanol sulphonate (5% w/v): sensitive culture.

Serological

(a) Growth inhibition tests were negative for M. mycoides subsp. mycoides, M. agalactiae subsp. agalactiae, M. capricolum, M. conjunctivae and Mycoplasma sp. (bovine group 7)*, but gave some inhibition in 2 of 3 tests with antisera to M. mycoides subsp. mycoides.

* except for a slight positive reaction in one test with one of two strains tested.

(b) Metabolic inhibition tests against the above species were negative, except that in one of two tests low titres were obtained against M. mycoides subsp. mycoides and M. sp. bovine group 7 (one strain only).

(c) Indirect fluorescent antibody tests were inconclusive, giving occasional positive results with some antisera to M. mycoides subsp. mycoides and capri and M. sp. (bovine group 7) but only negative results with M. conjunctivae and M. capricolum.

Conclusion The growth and biochemical characteristics of this strain are very similar to those of M. mycoides subsp. capri (and of the unnamed M. sp., bovine group 7) but the serological results may indicate a closer relationship with M. mycoides subsp. mycoides and appear to be consistent with the proposal that this strain belongs to Al-Aubaidi's mycoides-related caprine group 8, although this remains to be confirmed.

Dr. K.J. MacOwan, MRCVS,
Veterinary Research Laboratory,
P.O. Kabete,
KENYA.

18th November, 1975

Your Ref: Strain F30
(2OP; 6C)

Dr. R.H. Leach
REPORT for M.2054/75 - received 13.6.75 - F.D. ampoule.
Tests on this strain gave the following results:

**Biological**

- Growth conditions: On horse serum agar, growth dense after (several days) incubation in 5% CO₂/nitrogen & inferior in air.
- Glucose fermentation: positive (slight)
- Arginine utilization: negative
- Egg yolk agar: no "film & spots"
- Tetrazolium agar (aerobic): negative
- Serum liquefaction: negative
- Growth on serum-free agar: negative
- Sensitivity to 5% sodium polyanethol sulphonate: sensitive

**Serological**

(a) Growth inhibition tests against antisera for *M. mycoides* subsp. *mycoides*, *M. mycoides* subsp. *capri*, *M. agalactiae* subsp. *agalactiae*, *M. conjunctivae* & *M. capricolum* - all negative.

(b) Metabolic inhibition tests against available antisera for 20 fermentative Mycoplasma species*(plus Acholeplasma oculi, *M. bovigenitalium* & *M. agalactiae* subsp. *agalactiae* and *bovis*) all gave negative results.

**Conclusion**

The biological and serological characters indicate that this is a glucose-fermenting Mycoplasma species distinct from the recognised caprine and bovine mycoplasmas and from 12 other fermentative Mycoplasma species available for testing.

* Including those indicated in (a)

Signed: 
(Dr. R.H. Leach)

RHL: JW
Aarhus, 3rd February, 1976

Dear Dr. MacOwan,

We have today finished the examination of your strain F30 (our number C757).

Biochemical results:
- Digitonin sensitive
- Catabolism of glucose: +
- Catabolism of arginine: 0
- Phosphatase activity: 0
- Serum digestion: +
- Reduction of tetrazolium: +

The strain was identified, by growth inhibition, as

M. mycoides subsp. mycoides

reacting against PG1 antiserum as well as Y-goat antiserum (ovine/caprine group 8 of Al-Aubaidi). The examination of F38 is still not completed.

On behalf of the laboratory,

With best wishes,

Henning Ernø,
dr.med.vet.
International Collaborative Centre for Animal Mycoplasmas
Aarhus, 26th April, 1976

Dr. K.J. MacOwan  
Veterinary Research Laboratories  
P.O. Kabete  
KENYA, EAST AFRICA

Dear Dr. MacOwan,

I understand indeed that you are waiting for a report, and you may have our results so far, which probably will give the final conclusion that we are not able to identify your strain F38.

Biological examination:

1) Good growth after several days in candle jar on horse serum medium.

2) Glucose positive, arginine negative, phosphatase negative, serum digestion positive, sensitive to digitonin.  
   (note the difference here between our results and those of Dr. Leach).

Serological examination:

We have not been able to get positive reactions. It is not a M. mycoides subsp. mycoides, nor the caprine subspecies nor is it M. capricolum.

With best wishes,

Yours sincerely,

H. Ernø
The following two papers which have been published during the course of the study are included:—


The following paper has been published but the reprints are currently in the post to the Veterinary Research Laboratories, Kenya: MacOwan, K.J. and Minette, Jean E. (1976) A mycoplasma from acute contagious caprine pleuropneumonia in Kenya. Tropical Animal Health and Production, 8, 91 – 95.

Experimentelle intramuskuläre Infektion von Ziegen mit Mykoplasmen der chronischen Pleuropneumonie

Von K. Macowan, G. Kaliner und H. Krauss, mit 5 Abbildungen und einer Tabelle

Eingegangen am 15.1.1975

Material und Methoden

Für das Experiment I wurden zehn erwachsene, seit 18 Monaten in Isolation gehaltene Galakreuzungsziegen verwendet. Für das Experiment II wurden neun männliche Galeziegen im Alter von 4 bis 13 Monaten aus der Zucht- herde des Veterinary Research Laboratory benutzt. Die Herde war seit 3 Jahren frei von Krankheitserscheinungen.

Jede Ziege erhielt 1 ml Flüssigkultur mit $1.5 \times 10^9$ (Experiment I) koloniebildenden Einheiten (c.f.u.) oder $1.6 \times 10^7$ c.f.u. (Experiment II) des Mykoplasmenstammes F 50 in die Hinterbackenmuskulatur inokuliert. Der Stamm war 10mal geklonnt worden und lag in der 15. Flüssigkulturpassage vor.

Das Inokulum wurde vor der Inokulation bakteriologisch überprüft. Als Kontrollen dienten insgesamt 9 weitere Ziegen, die die gleiche Menge sterile Flüssigkultur inokuliert bekamen. Medien und Kulturverfahren sind an anderer Stelle beschrieben worden (Macowan, 1975).

Proben-Entnahme

Im Experiment I wurde je ein Tier am 3. und 4. außerdem am 25. Tag post infectionem (p. i.) durch intravenöse Inokulation von 3 ml Euthanal [Mar und Barke Ltd.] getötet.
und enthielt und Proben für die histologische und elektronenmikroskopische Untersuchung entnommen. Die übrigen Tiere wurden — bis auf ein Tier — bis zum Exitus beobachtet und anschließend histologisch und kulturell untersucht. Eine überlebende Ziege stand 18 Monate unter Beobachtung.

In Experiment II wurden alle Tiere nach folgendem Schema geötet, enthielt und Proben für kulturelle, histologische und elektronenmikroskopische Untersuchung entnommen:

- 1. Tag: 1 Ziege
- 2. Tag: 2 Zeien
- 3. Tag: 3 Zeien
- 4. Tag: 2 Zeien
- 5. Tag: 1 Ziege

Kulturell wurden jeweils folgende Proben auf Mykoplasmen und bakterielle Sekundärinfektion untersucht:

- poplitealer, inguinaler, iliacaler und subiliacaler Lymphknoten der affizierten Seite, ebenso die bronchialen Lymphknoten.
- Exsudate: ödematöse Flüssigkeit und soweit vorhanden Blut.


Für die elektronenmikroskopische Untersuchung wurden sofort nach der Tötung Proben aus verändertem Muskelgewebe, normale Muskulatur, Teile der iliakalen Lymphknoten, Leber und Milz entnommen und 2 Stunden mit 5% Glutaraldehyd in 0,2 M Cadycatplatterfiexiert. Außerdem wurden einzelne, auf festen Nährböden gezüchtete Kolonien des verwendeten Mykoplasmastammes aus dem Agar ge- stanzt und im gleichen Medium 1 Stunde fixiert.

Alle Proben wurden 2—5 Stunden mit 1% Osmium in 0,2 M Cadycatplatter nachfixiert. Die Präparate wurden anschließend kurz in 0,2 M Natrumazetat gespült, 20 Min. mit 0,5% Uranylzetaet behandelt und wieder kurz mit Natriumazetat gespült. Die Dehydration erfolgte mit Aceton. Anschließend wurden die Proben im Durcupan (Fluka, Basel) eingebettet. Ultraßdinschnitte (60—100 nm) wurden auf einem Reichert Ultramikromot OMU2 angefertigt, diese nochmals mit Uranylzetaet behandelt und mit Bleizitrat kontrastiert und in einem Zeiss Elektronenmikroskop EM 802 untersucht.

Ergbnisse

Das Experiment I diente als Vorversuch. Da die pathomorphologischen Veränderungen bei den Tieren aus beiden Versuchen ähnlich waren, werden hier nur die von Experiment II abweichenden Befunde beschrieben.


Die restlichen zwei Zeien waren am 3. und 4. Tag p. i. für die histologische und elektronenmikroskopische Untersuchung getötet worden.

Mykoplasmen (Stamm F30) wurden aus allen veränder-ten Geweben aller Ziegen reisoliert.

Im Experiment II stieg bei den infizierten Ziegen die Körpertemperatur innerhalb von 2—3 Tagen auf 40°C. Gleichzeitig entwickelte sich im Bereich der Inokulationsstelle eine umfangreiche Schwellung. In den folgenden Tagen zeigten die Tiere eine immer stärker werdende Lähmung, Ataxie und Körpertemperatur über 41,5°C. Die Schwellung dehnte sich bis zum Sprung- bzw. Fersenbein aus.


Elektronenmikroskopisch konnten in den verändert-ten Geweben je nach Lokalisation der Zellen die verschieden-sten Stadien der Zellschädigung wie Schwellung der Mitochondrien mit Desorganisation der Cristae, Vakuolierung...
Tabelle 1: Reisolation von Mykoplasmen (Stamm F 30) aus verschiedenen Organen experimentell infizierter Ziegen (Experiment II)

<table>
<thead>
<tr>
<th>Organ</th>
<th>1. Tag (1 Tier)</th>
<th>2. Tag (2 Tiere)</th>
<th>3. Tag (3 Tiere)</th>
<th>4. Tag (2 Tiere)</th>
<th>5. Tag (1 Tier)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pol. LK</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>amin. LK</td>
<td>n. u.</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Blac. LK</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Sulbi. LK</td>
<td>n. u.</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Bronc. LK</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Muskel</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Milz</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Niere</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Leber</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Lunge</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Gehirn</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Myocard</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Blut</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Exsudate</td>
<td>n. u.</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

1 = Reisolation von Mykoplasmen gelungen
2 = Reisolation von Mykoplasmen nicht gelungen
- = nicht untersucht


In 4 Tage alten Kolonien des Stammes F 30 auf festen glukosehaltigen Nährböden konnte wir sog. rho-Formen (Petersson u. Mitarb., 1973) beobachten (Abb. 5). Ihr Nadirweis gelang nicht in vivo. Im übrigen waren keine morphologischen Unterschiede an den Organismen in vivo und in vitro feststellbar.
mit Mykoplasma mycoides var. capri in Kulturen von Hühnerembryonsträgen die Produktion von Wasserstoffperoxydi ist. Der Nachweis der Produktion von H₂O₂ bei vielen anderen Mykoplasmen, auch bei avirulenten Stämmen, lädt jedoch vermuten, daß noch andere Faktoren eine Rolle spielen, die zur Schädigung des Wirtsgewebes führen und die evtl. dem H₂O₂ oder anderen Toxinen das Eindringen in die Wirtszelle ermöglichen (Collier, 1969).


Weiterhin wurden in dieser Richtung vonnöten neue Ergebnisse hinsichtlich der Pathogenese dieser Infektion erwähnt.

Zusammenfassung
Mit einem in Kenia aus Zeigen mit chronischer Pleuro-Pneumonie isolierten geklonnten Mykoplasmenstamm wurde in Versuchsziegen durch i.m. Inokulation eine nekrotisierende Myositis erzeugt, die durch leukozytäre Infiltrate gekennzeichnet war. Elektronenmikroskopisch wurden Mykoplasmen in großer Zahl in nekrotischem Gewebe, zwischen noch intakten Muskelbündeln und intrazellulär in polymorphkernigen Leukozyten nachgewiesen. Schon am 1. Tag p.i. kam es zur Generalisierung des Erregers; Mykoplasmen (Stamm F30) wurden aus den meisten, ab dem 2. Tag p.i. aus allen untersuchten Geweben reisoliert.

K. MacOwan, G. Kaliner und H. Krauss: Experimental intramuscular injection of a Mycoplasma of Chronic Caprine Pleuropneumonia

Summary
A necrotic myositis was produced in goats after i.m. inoculation of a cloned strain of Mycoplasma isolated from caseous Chronic Caprine Pleuropneumonia. It was characterized by infiltration with polymorphonuclear leukocytes. The infection was found in large numbers in necrotic tissue, also between intact muscle fibres and within polymorphonuclear leukocytes. The infection generalized already within 24 hours p.i. The agent (strain F30) was reisolated from most, and from the second day on, from all tissues investigated.

Literaturverzeichnis


Diskussion


Abb. 5. Rho-Form in einer Kolonie von Mykoplasmen des Stammes F 30 auf festem glutkholigem Nährboden. Vergr. 34 695 ×

Anschrift der Verff.: K. MacOwan und Dr. G. Kalinier, Veterinary Research Laboratories, P.O. Kabete, Kenya. Prof. Dr. H. Krauss, Abteilung Zoonosen, Institut für Hygiene und Infektionskrankheiten der Tiere, 63 Gießen, Frankfurter Str. 89.
A MYCOPLASMA FROM CHRONIC CAPRINE PLEUROPNEUMONIA IN KENYA
K. J. MACOWAN
Veterinary Research Laboratories, Kabete, Kenya

SUMMARY
A new mycoplasma was isolated from cases of chronic caprine pleuropneumonia in Kenya. It belonged to the species Mycoplasma mycoides being a member of Al-Aubaidi's Group 8. When inoculated into goats and sheep the organism caused pleuropneumonia and local subcutaneous lesions. The pleuropneumonia was not contagious. In contrast the organism was non-pathogenic in cattle.

INTRODUCTION
Contagious caprine pleuropneumonia has a wide geographic distribution. There is confusion as to the nature of the causal mycoplasma; recent studies have segregated the mycoplasma into those that cross reacted by growth and metabolic inhibition test with M. mycoides subspecies mycoides and those like the PG3 strain of M. mycoides subspecies capri (Al-Aubaidi, Dardiri and Fabricant, 1972) that did not. In Kenya, the disease has been recognised for over 40 years (Mettam, 1929) but, to date, the causal mycoplasma has not been identified. An organism isolated from chronic cases of caprine pleuropneumonia in Kenya was, therefore, studied culturally and serologically and its pathogenicity for domestic ruminants examined.

MATERIALS AND METHODS

Mycoplasmas
The isolated organism was designated F30. It was purified by serial subinoculation of a single colony 10 times, three times on media without bacterial inhibitors.

Known mycoplasmas used comparatively were the PG3 strain (National Type Culture Collection 10137) of contagious caprine pleuropneumonia, the Nigerian reference strain N108 (Cottew and Leach, 1969) and the Gladysdale strain of M. mycoides subspecies mycoides.

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 and kept in isolation for 6 months prior to experiment. Experimental groups to be infected and control groups were balanced for breed, sex and age.

Inocula
All infective inocula were 24–48 h log phase broth cultures of F30. Mycoplasma cultures for inoculation were confirmed free of contaminating bacteria and their titres ranged from $6 \times 10^9$ colony-forming units per ml (cfu/ml) to $3.2 \times 10^9$ cfu/ml.

Cultural examination of specimens
All specimens were plated on solid mycoplasma medium and stored at 4°C. Those specimens showing no colonies within 24 h incubation at 37°C were macerated.
in a minimum volume of mycoplasma broth and inoculated in 10-fold dilution from 10^4 to 10^6 in broth medium. Mycoplasma reisolates from three cases in each experiment were tested with growth-inhibiting serum prepared to F30 in rabbits.

All specimens were also culturally examined for bacteria.

Experiments

1. **Pathogenicity for goats by the intratracheal-endobronchial route of inoculation**

Eight goats of the Galla and crossbred Galla type, ranging from 9 to 18 months in age received 5 ml of F30 broth culture and 5 ml of sterile broth via the intratracheal endobronchial route (Abdulla and Lindley, 1967). A further four goats were kept in close contact with the inoculated animals. A control group of eight goats was inoculated similarly with 10 ml of sterile broth. The experiment was terminated after 54 days, 40 days following the death of the last affected goat.

2. **Pathogenicity for goats by contact transmission**

Eight crossbred Galla goats aged 5–8 months were maintained in close contact with an acute experimental pleuropneumonia case initiated with F30. Following the death of the affected animal another was introduced. The contact animals were exposed to inoculated and affected goats for 66 days, and they were killed 42 days later.

3. **Pathogenicity for sheep**

Eight adult sheep of the Down and Merino breeds were inoculated with 1 ml of F30 broth culture and 9 ml of sterile broth via the intratracheal route and four others were kept in close contact. The control group contained the same number of animals, eight of which were inoculated in this way with sterile broth. Survivors were slaughtered 60 days post-inoculation (p.i.), 43 days after the death of the last clinically affected sheep.

4. **Pathogenicity for cattle**

Two 5–7 month old Jersey calves were inoculated intratracheally with 100 ml of F30 culture and two control calves received a similar volume of sterile broth by this route. The calves were slaughtered 69 days post inoculation.

Four 4–8 month old crossbred Ayrshire calves were inoculated with 3 ml of F30 culture subcutaneously behind the shoulder and four control calves were treated similarly with sterile broth. Two inoculated calves were slaughtered 28 days p.i. and two were killed 103 days p.i.

Cultural methods

Media

Newing's tryptose broth was employed (modified by Gourlay, 1964). Solid medium containing 30 per cent (v/v) unheated pig serum was prepared by the addition of 2 per cent (w/v) agar to the broth. Phenol red was added to both liquid and solid media to a final concentration of 0.001 per cent (w/v). For the growth of caprine mycoplasma strains, glucose was reduced to 0.2 per cent (w/v). All cultures were incubated aerobically at 37°C except when stated otherwise.
Fermentation tests

Log phase cultures of all strains were inoculated into broths containing 1 per cent (w/v) of one of 20 standard substrates: glucose, laevulose, mannose, maltose, trehalose, starch, dextrin, glycogen, sorbitol, arabinose, xylose, rhamnose, adonitol, galactose, mannitol, dulcitol, sucrose, raffinose, inulin and salicin. Concurrently each organism was inoculated into broth without sugar and broth without serum and an equal number of inoculated and uninoculated broths were set up. All inoculated broths received 1 part by volume of culture to 9 parts of broth. Before inoculation and daily thereafter the pH of each broth was measured with a pH meter. After 7 days the experiment was terminated and all broths checked for the absence of contaminating bacteria.

Other cultural tests

Two methods for detecting haemolysis were used; in the first organisms were grown on solid medium without thallium acetate, containing 5 per cent (v/v) defibrinated blood, either horse, goat, sheep, guinea pig, or chicken (Cottew, Watson, Erdag and Arisoy, 1969). In the second well-separated, young colonies were thinly overlaid with 1 per cent (w/v) PPLO agar (Difco) containing 5 per cent (v/v) of the same defibrinated bloods before reincubation.

The organisms were tested for production of peroxide (Cole, Ward and Martin, 1968) on Newing’s tryptose agar containing horse red blood cells and no thallium acetate. Inocula were prepared by scraping young confluent colonies from solid medium. Edward’s technique (1950) was used to test for methylene blue reduction and the liquefaction of inspissated medium was tested by the method detailed by Jonas and Barber (1969) using Newing’s tryptose broth including either 25 per cent (v/v) goat serum, 30 per cent (v/v) bovine serum, or 30 per cent (v/v) pig serum, instead of the usual serum supplement. Haemagglutination and haemadsorption tests were performed using complement fixation test antigen and Newing’s tryptose agar respectively (Manchee and Taylor-Robinson, 1968). In both tests goat and guinea-pig red blood cells were collected in Alsever’s solution and washed in veronal buffer, which was also used as diluent and for washing colonies.

Culture in embryonated eggs

Four 7-day embryonated hens’ eggs were inoculated via the yolk sac with 0.1 ml of F30 log phase culture. Four similar eggs were inoculated with sterile broth and four others maintained as control eggs. Fourteen passages were made using aspirated yolk sac fluid as the inoculum. Inocula and harvests were examined for the presence of contaminating bacteria and the harvest from the fourteenth passage was grown in broth and tested in the growth-inhibition test with F30 antiserum.

Complement-fixation and agar gel diffusion tests

Antigens

Organisms were cultured in 2-litre volumes of Newing’s tryptose broth containing 10 per cent (v/v) rabbit serum which had been heated at 56°C for 0.5 h. When the titre exceeded 10⁹ colony forming units per ml (cfu/ml) the culture was ultracentrifuged at 40 x 10³ rpm (1 h) at 5°C. The deposit was resuspended and washed three times in physiological saline prior to final suspension in 20 ml of physiological saline and storage at -20°C. Sterile broth treated as above constituted the sediment antigen while the second control antigen was freeze-dried medium reconstituted at 200 mg/ml.
Antisera

Hyperimmune rabbit antisera were prepared by a modification of the method of Krauss and Wandera (1970), in which complete Freund's adjuvant replaced incomplete Freund's adjuvant. The sera of rabbits to be immunised were confirmed negative by the tests for which they were prepared.

Other reagents included two bovine sera from field cases of contagious bovine pleuropneumonia with a known high complement-fixation titre to M. mycoides subspecies mycoides.

For all serological tests rabbit and bovine sera were inactivated at 56°C for 0.5 h, while goat sera were inactivated for the same period at 60°C.

Agar gel diffusion test technique

Molten 2 per cent (w/v) agarose was mixed with an equal volume of double-strength barbitone buffer, pH 8.2 (Kohn, 1968) and 3 ml volumes pipetted on to agar-coated standard microscope slides. Well patterns with one central and six peripheral wells 7 mm apart and 4 mm in diameter were prepared. All antigens were frozen and thawed six times in an acetone and dry ice bath. Precipitation lines were read after 48 h at room temperature in a moist chamber.

Complement-fixation test technique

Microtitre plate tests were performed by the method of Grist, Ross, Bell and Stott (1966) using 0.025 ml volumes, two volumes of complement containing six minimum haemolytic doses and 1.5 per cent (v/v) final concentration of red blood cells.

When the complement-fixation test was used to examine goat sera only three minimum haemolytic doses of complement were employed.

Growth inhibition tests

For preparation of growth inhibiting rabbit antisera the first three injections were as described previously and thereafter rabbits were inoculated weekly with 1 ml of high titre culture intravenously.

The method employed was a modification of the procedure described by Clyde (1964). Serum wells 6 mm in diameter were used instead of filter paper discs soaked in serum.

RESULTS

Clinical observations

Three field cases from two goat herds which had suffered from an acute outbreak of contagious caprine pleuropneumonia several months previously were studied. The cases were emaciated, and two were suffering from a relapse associated with high body temperature and pneumonic symptoms. Post-mortem examinations revealed extensive fibrous pleuropneumonia. The lung lesions enclosed areas of grey to white necrotic tissue varying from 1–2 mm to several centimetres in diameter and in two cases the affected lung tissue enclosed a 2–4 cm diameter, heavily encapsulated abscess.

The lung lesions of two cases yielded Pasteurella haemolytica and Pasteurella multocida, while those of the third yielded Pasteurella haemolytica alone. In addition, abundant, rapidly growing mycoplasma were isolated from all three cases.

The isolate from one case, F30, was chosen for further study. Using antiserum prepared to F30 isolates from all three cases were shown to be the same by growth inhibition tests.
Experimental results

Goats inoculated by the intratracheal endobronchial route showed acute pneumonia symptoms within 2–5 days, and where it occurred swelling about the site of inoculation by 3 days. Body temperature rose sharply up to 42°C remaining high or falling 1–2 days before death 3–14 days post-inoculation.

All animals infected by the intratracheal endobronchial route developed lung lesions. Four goats suffered severe lung lesions which replaced one-sixth to one-third of the total lung tissue and only small subcutaneous lesions about the site of entry of the endobronchial tube in the trachea. The other four cases showed smaller focal lung lesions but extensive lesions around the trachea. Terminally, six of the affected goats developed mild diarrhoea.

Affected lung tissue was red to grey, oedematous in section and similar in texture to liver tissue, giving the lesion a well-defined margin. In two cases isolated areas with enlarged, oedematous, interlobular septae were observed and in three cases the centre of larger lung lesions was paler, more friable and somewhat necrotic. The extensive lung lesions were covered with fibrin of varying thickness forming adhesions, and clear pleural fluid was also present. The bronchial and mediastinal lymph nodes from all inoculated goats were grossly enlarged, being oedematous and haemorrhagic in section.

The six goats which developed mild diarrhoea terminally had congestion in parts of the large intestine mucosa and enlargement of the mesenteric lymph nodes. In five cases the spleen was enlarged and in one the splenic capsule was thickened with fibrin, showing multiple haemorrhages. In addition the thymus of two affected goats was enlarged showing prominent septae distended with oedema and fibrin, while in another case acute fibrinous epicarditis was present.

The subcutaneous lesions about the trachea exuded oedema fluid containing fibrin that distended the connective tissue and muscle fascia. The subcutaneous connective tissue and superficial muscle fascia showed numerous deposits of yellow fibrin, petechiae and ecchymoses.

All the inoculated goats yielded abundant growth of mycoplasma, culturally and morphologically similar to F30, from the spleen, liver and lungs, in addition to all the lesions mentioned above. One of them was 4 months pregnant and although no macroscopic lesions were present in the foetus or foetal membranes, mycoplasma resembling F30 were isolated from the foetal fluids.

None of the in-contact goats became clinically affected and no lesions were observed at post-mortem examination. In addition no mycoplasma similar to F30 were isolated from specimens of lung, liver, kidney, spleen, bronchial lymph node, mediastinal lymph node and retropharyngeal lymph node.

However, all the in-contact goats developed complement-fixing antibodies to F30 ranging in titre from 1/16 to 1/64.

Three of the eight inoculated sheep died between 9 and 17 days p.i. Two of these cases developed extensive lesions about the site of inoculation as well as acute fibrinous pleuropneumonia. The third case showed only an extensive subcutaneous tracheal lesion. No symptoms were observed in the other inoculated sheep although at post-mortem examination the lungs of two revealed fibrous pleuropneumonia. The in-contact sheep remained unaffected and no lesions were observed at post-mortem examination.

From the tissues of the fatal cases mycoplasma similar to F30 were isolated from the lung, liver, spleen, and kidney, in addition to the lesions mentioned above. These organisms were also isolated from the chronic lung lesions of one of the killed sheep.

No mycoplasma were isolated from lung, liver, spleen, or kidney specimens from other...
sheep in the infected group. From the chronic lung lesions found in the two slaughtered sheep Staphylococcus species, Pseudomonas species, and Corynebacterium pyogenes were isolated.

The two calves inoculated by the intratracheal route showed no symptoms and no lesions were observed at post-mortem examination. No mycoplasma were isolated from lung, liver, spleen and mediastinal lymph node specimens or from the tissue about the site of inoculation.

Following subcutaneous inoculation the four calves were similarly unaffected. Post-mortem examination of two calves 28 days p.i. revealed small areas of mild haemorrhage at the site of inoculation, while the prescapular and axillary lymph nodes of that side were somewhat enlarged.

In the last two cases, killed 103 days p.i., the axillary and prescapular lymph nodes draining the site of inoculation were also enlarged. With the exception of a calf killed 103 days p.i. mycoplasma resembling F30 were isolated from the axillary lymph node draining the site of inoculation and in two cases from the prescapular lymph node of the inoculated side.

None of the calves developed complement-fixing antibodies to F30 antigen or M. mycoides subspecies mycoides (Gladysdale) antigen.

All mycoplasma reisolates tested with F30 growth-inhibiting serum were totally inhibited.

Cultural characteristics

Colonies of the reference strains and F30 were similar although M. mycoides subspecies mycoides (Gladysdale) colonies grew more slowly. Wet preparations of broth cultures of the caprine strains differed from those of M. mycoides subspecies mycoides (Gladysdale) which showed much longer, more elaborate filaments.

F30, PG3 and N108 lowered the pH of media containing glucose, laevulose, mannose, maltose, trehalose, starch, dextrin and glycogen by 1-8 or more. The pH of the other media fell by one or less, with the exception of cultures grown in medium containing sorbitol. The pH of the last medium was lowered by 1-6-2-3, depending on the strain tested. The pH of cultures grown in medium without added carbohydrate fell by 0-7-0-75. M. mycoides subspecies mycoides (Gladysdale) fermented these carbohydrates in the same way with the exception that the pH of cultures containing laevulose and sorbitol did not fall by more than 1. The pH of the culture grown in medium without added carbohydrate fell by 0-4.

Clear haemolysis with a light brown or yellow border was observed with all strains and species of red blood cells. All organisms showed production of peroxide by black discoloration of the benzidine blood agar plates within 24-48 h. Under anaerobic conditions no production of peroxide occurred and only very slight haemolysis. All organisms reduced methylene blue within 3-4 days. Inspissated medium containing goat and bovine serum was liquefied by all strains; no liquefaction of inspissated medium containing pig serum occurred with any of the organisms. Liquefaction was observed within 24-48 h with strains F30, PG3 and N108 but by day 4 only slight liquefaction was seen with M. mycoides subspecies mycoides (Gladysdale). No haemagglutination or haemadsorption was observed with any of the strains.

F30 grew vigorously in embryonated eggs, which died 2-3 days post-inoculation. Organisms harvested from the fourteenth passage were inhibited by growth-inhibiting serum prepared to F30.
Serological findings

The results of the agar gel diffusion tests indicated that each strain had at least three antigens in common with any one of the others. Homologous reactions showed four to six precipitation lines.

Assuming a four-fold difference of complement-fixation test titre to be significant, all hyperimmune rabbit sera reacted to the same titre with *M. mycoides* subspecies *mycoides* (Gladysdale). Each of the caprine strains showed a much lower titre with *M. mycoides* subspecies *mycoides* (Gladysdale) hyperimmune rabbit serum than with their homologous serum. The clinical bovine sera confirmed the specificity of the hyperimmune rabbit serum to *M. mycoides* subspecies *mycoides* (Gladysdale). Although all four strains were related by complement fixation test, the relationship between PG3 and N108 was particularly close.

The growth-inhibition test results indicated F30 to be the same as *M. mycoides* subspecies *mycoides* (Gladysdale). Both F30 and *M. mycoides* subspecies *mycoides* (Gladysdale) antisera inhibited F30 and *M. mycoides* subspecies *mycoides* (Gladysdale) colonies but neither affected colonies of PG3 or N108.

**DISCUSSION**

F30 was pathogenic, causing fibrinous pleuropneumonia, connective tissue lesions and muscle lesions in goats and sheep following experimental inoculation. In these respects the organism closely resembled agents of contagious caprine pleuropneumonia (CCPP) described by Longley (1940, 1951) and Shirlaw (1949). The experiments with F30 were of particular significance because of the use of animals either reared in isolation or maintained in isolation for 6 months prior to experiment.

The enteric lesions were not without precedent; Longley (1951) described diphtheritic lesions in the small intestine of goats following intravenous inoculation. The distribution of reisolates in all inoculated goats suggested mycoplasmal pneumonia; Shirlaw (1949) made a similar observation.

*M. mycoides* subspecies *capri* has been recorded to infect the foetuses of experimentally inoculated sheep (Longley, 1951) and goats (Yedloutschnig, Taylor and Dardiri, 1971); similarly *M. mycoides* subspecies *mycoides* may cross the placenta in cattle (Stone, Masiga and Read, 1969) and in sheep (Beller and Tahssin-Bey, 1926). The inoculation of cattle with agents of CCPP or crude exudates from field cases of this disease has given variable results. Barber and Yedloutschnig (1970) and Provost (1966) report fatal infection while Lindley and Abdulla (1969), Longley (1951), Mettam (1929) and Shirlaw (1949) record no infection.

Several authors (Longley, 1940, 1951; Mettam, 1929; Shirlaw, 1949) demonstrated in-contact transmission of the natural disease and the disease induced with exudates and extracts of lesions from field cases. Apart from Yedloutschnig *et al* (1971) there was no record of in-contact transmission of disease induced by cultured organisms. It is noteworthy that while the latter authors achieved this result with organisms classified as *M. mycoides* subspecies *capri* these were not isolated from the classical disease.

That F30 initiated a complement-fixing antibody response in contact goats indicated this agent could infect by contact but under the experimental conditions it failed to cause disease. It may be that F30 can cause contagious disease when goats are subjected to environmental stress factors. However because the organism has been isolated from only three chronic caprine pleuropneumonia cases it is unlikely to be the cause of contagious caprine pleuropneumonia in Kenya.

Recently agents classified as *M. mycoides* subspecies *capri* were divided into those related to *M. mycoides* subspecies *mycoides* by growth inhibition test (Group 8)
and those which were not (Al-Aubaidi et al, 1972). The morphological and cultural characteristics together with the findings of complement-fixation and agar gel diffusion tests placed F30 in the species *M. mycoides*. Further the growth-inhibition test results indicated F30 belonged to Group 8.

**CONCLUSION**

A mycoplasma isolated from chronic caprine pleuropneumonia cases in Kenya was found experimentally pathogenic for goats and sheep but not for cattle. The nature of the experimental disease suggested a strain of *M. mycoides* subsp. *capri*, although in-contact transmission of the experimental disease between goats was not successful.

Morphological, cultural and serological findings placed F30 in the *M. mycoides* species and the growth inhibition test also indicated F30 to be a member of Al-Aubaidi's Group 8.

**ACKNOWLEDGEMENT**

This paper is published by kind permission of the Director of Veterinary Services, Kenya.

Accepted for publication April 1975

**REFERENCES**


UN MYCOPLASME PROVENANT D'UNE PLEUROPNEUMONIE CHRONIQUE CAPRINE AU KENYA

Résumé—Un nouveau mycoplasme a été isolé à partir de cas de pleuropneumonie caprine chronique au Kenya.

Il appartient à l'espèce M. mycoides, membre du Groupe 8 d'Al Aubaidi. Inoculé à des chèvres et des moutons, ce germe cause une pleuropneumonie et des lésions sous cutanées locales. La pleuropneumonie n'est pas contagieuse. Par contre, le germe n'est pas pathogène pour les bovins.

UN NUEVO MICOPLASMA DE LA PLEURONEUMONIA CAPRINA CRONICA EN KENIA

Resumen—Se aisló un nuevo micoplasma de casos clínicos de pleuroneumonía caprina crónica en Kenia. El agente pertenece a la especie Mycoplasma mycoides, siendo un miembro del grupo 8 Al-Aubaidí. El organismo produjo pleuroneumonía lesiones subcutáneas cuando se inoculó en cabras y ovejas. La enfermedad producida no fue contagiosa y el organismo no fue patógeno para los bovinos.
REFERENCES

The Veterinary Record, 78, 667-668.


Biochemical characterisation and antigenic relationship of Mycoplasma mycoides subspecies mycoides Freundt and 
Mycoplasma mycoides subspecies capri (Edward) Freundt. 
International Journal of Systematic Bacteriology, 22, 155-164.

The Cornell Veterinarian, 58, 555-571.

Anon (1964) Immunology. Relationship to other mycoplasma. 
Second meeting of the FAO/OIE/CCTA expert panel on contagious bovine pleuropneumonia, p.7 Muguga, Kenya.


The Journal of General Microbiology, 55, 45-58.


*Infection and Immunity, 2*, 431-438.


Clyde, W.A. (1964) Mycoplasma species identification based upon growth inhibition by specific antisera. 
*Journal of Immunology, 22*, 958-965.

*Journal of Bacteriology, 95*, 2022-2030.


Journal of Comparative Pathology, 72, 541-551.


Debonera, G. (1937) Une forme particuliere et grave d'agalaxie: la maladie des oedemes des chevres de Sparte.


Journal of Bacteriology, 88, 727-744.

The Journal of General Microbiology, 4, 311-329.

Edward, D.G.ff (1953) Organisms of the pleuropneumonia group causing disease in goats. The Veterinary Record, 65, 873-875.


I. Examination of body fluids from cases of contagious bovine pleuropneumonia.
Research in Veterinary Science, 5, 473-482.

The Journal of Medical Microbiology, 3, 111-123.

The Journal of General Microbiology, 57, 131-142.


Hakioglu, F. & Bogrun, O. (1958) Myositis in goats infected experimentally with the agent of caprine contagious pleuropneumonia.

Wiener Tierarztliche Monatschrift, 40, 402-413.


Zentralblatt fur Veterinarmedizin. In press.

Bulletin of Epizootic Diseases of Africa, 6, 277-278.


The Journal of General Microbiology, 12, 95-99.
Kohn, J. (1968) Cellulose acetate electrophoresis and immuno-
diffusion techniques. In I. Smith (ed). Chromatographic

Krauss, H. & Wandera, J.G. (1970) Isolation and properties of
mycoplasma from the respiratory tract of sheep with
Jaagsiekte in Kenya.
Journal of Comparative Pathology, 80, 389-397.

Krogsgaard-Jensen, A. (1972) Mycoplasma: Growth precipitation
as a serodiagnostic method.
Applied Microbiology, 23, 553-558.

Laws, L. (1956) A pleuropneumonia-like organism causing peritonitis

Lemcke, R.M. (1964) The serological differentiation of mycoplasma
strains (pleuropneumonia-like organisms) from various sources.

Lemcke, R.M. (1965) A serological comparison of various species of
mycoplasma by an agar gel double diffusion technique.
The Journal of General Microbiology, 38, 91-100.

Journal of Bacteriology, 110, 1154-1162.


PG3 the type strain of Mycoplasma mycoides subspecies
capri.
Research in Veterinary Science, 16, 119-121.


Longley, E.O. (1951) Contagious caprine pleuropneumonia. 

Tropical Animal Health and Production - 8, 91-95.


Journal of Bacteriology, 96, 1867-1869.


Pillai, C.P. (1965) Studies on pneumonia of goats in the Sudan. Thesis presented to the Royal College of Veterinary Surgeons, United Kingdom, for the Diploma of Fellowship of the Royal College of Veterinary Surgeons (FRCS).


Smith, G.R. (1965) **Infection of small laboratory animals with Mycoplasma mycoides var. capri and Mycoplasma mycoides var. mycoides.** The Veterinary Record, 77, 1527-1528.

Smith, G.R. (1967) **Experimental infection of mice with Mycoplasma mycoides var. capri.** Journal of Comparative Pathology, 77, 21-27.

Smith, G.R. (1969) **A study of Mycoplasma mycoides var. mycoides and Mycoplasma mycoides var. capri by cross-protection tests in mice.** Journal of Comparative Pathology, 79, 261-265.


Tessler, J. (1973b) Differentiation among strains of goat mycoplasma by incident light immunofluorescence. Canadian Journal of Comparative Medicine, 37, 405-408.


