# STUDIES ON
# THE BACTERIAL SPECIES ASSOCIATED WITH
# PNEUMONIA IN GOATS


By

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THESIS

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# D E C L A R A T I O N

I hereby declare that this thesis entitled "STUDIES ON THE BACTERIAL SPECIES ASSOCIATED WITH PNEUMONIA IN GOATS" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title of any other University or Society.

R. Madhusoodanan Pillai.

Mannuthy,

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# C E R T I F I C A T E

Certified that this thesis, entitled "BACTERIAL SPECIES ASSOCIATED WITH PNEUMONIA IN GOATS" is a record of research work done independently by Sri. R. Madhusoodanan Pillai under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Mannuthy,
31---1---1977.

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C O N T E N T S



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# INTRODUCTION

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Respiratory diseases often take a heavy toll of the lives of goats, particularly the young ones. Among the diseases of the respiratory system, pneumonia both as a primary and a secondary affection has a major role in causing mortality among goats.

There is dearth of knowledge regarding the incidence of pneumonia of goats in India. No systematic investigation appears to have been made to delineate the factors associated with its aetiology under the conditions of husbandry of goats in our country. However, some information is available on the incidence of this disease syndrome among goats in various parts of our country. Minett (1950) reported that the incidence of pneumonia was highest (39.3%) in goats, under sixth month of age. Tiwari and Pandit (1964) found that 13% of 1385 goats and sheep examined at four abattoirs in Madhyapradesh had pneumonia. Further reports on the Survey of incidence of goat-pneumonia were based on the observations made at abattoirs and routine necropsies carried out by Ramachandran and Sharma (1969). Out of the 1755 goats necropsied, 507 revealed lung lesions, the incidence being 28.22%. This conclusion is in general agreement with the observations on disease problems of goats in other countries of the world, where pneumonia is the most important

problem affecting the economics of goat production (Tiwari and Pandit, 1967; Pearson et al. 1972; Macowan and Minette, 1976).

Despite the general similarity in the clinical picture, it is becoming increasingly evident that the aetiology of respiratory infections in goats is very complex. Under intensive conditions of management physical stresses in addition to infectiousagents are gnnerally considered as of special significance. Physical stress as from exhaustion, over crowding and irregular feeding is an important factor in lowering the resistance and thereby enabling infectious agents to precipitate the disease (Mckercher,1964). Evidence is also accumulating which indicates that the severe clinical disease seen under field conditions isthe result of interactions of two or more infectious agents under the influence of physical stresses.

In the goat farm attached to the Kerala Agricultural University, large numbers of goats are raised under ideal managemental and hygienic conditions, but very often losses due to pneumonia have been encountered especially in young animals. Limited studies conducted at the Department of Bacteriology have revealed occurrence of various species of organisms associated with such conditions. However, a detailed investigation on the aetiological factors has not

so far been carried out. Hence this thesis is directed into three aspects in the following order:-

1. Studies on the bacterial species associated with pneumonia in goats based on their morphological, cultural and biochemical characters.

2. Studies on the pathogenicity of the isolates to experimental animals as well as to the primary host.

3. Studies on the drug sensitivity of the isolates to the common therapeutic agents used in human and animal practice.

# REVIEW OF LITERATURE

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## History and incidence

Pneumonia is the inflammation of pulmonary parenchyma and is usually accompanied by the inflammation of bronchioles. The exposure of lungs to the external environment via the upper alimentary and respiratory tracts and to the internal environment via the blood vessels, predisposes animals to the respiratory diseases (Jubb and Kennedy, 1970). Among the various respiratory diseases, pneumonia is considered to be a very serious problem encountered in animals (Barr et al. 1951; Greig, 1955; Pounden et al. 1956; Gowan et al. 1957; Klemene et al. 1964; Stamp, 1968; Mohn and Utklev, 1974; Sarkar and Bhattacharyya, 1975; Thomas et al. 1975).

There are many reports on the incidence of pneumonia in goats, sheep and cattle in India and abroad. Langham, et al. (1944) reported that the incidence of pneumonia in sheep in Michigan State was 32 to 33%. The authors obtained the above data from the record of necropsies conducted at Michigan State Veterinary College for a period from 1912 to 1941. They also studied correlation of age and season with the incidence of pneumonia. The incidence was found to be high in the first sixty days of life and more percentage of cases was

recorded in winter and spring. St. George (1972) reported the incidence of pneumonia in sheep inSouth East Australia and recorded that 75 percent of deaths were under one year of age. Similar study in lambs conducted by Korikov (1974) in Soviet Union revealed that there was a seasonal variation in the prevalence of pneumonia. He had reported that 30 to 40 percent of pneumonic cases were recorded during summer season while the incidence went up to 70 percent during winter and spring. Murgera and Kramer (1967) stated that the incidence of pneumonic death in goats in Kenya was higher (60%) when they were exposed to stress.

Reports regarding the incidence of pneumonia in goats in India are available. Minette (1950) reported that the mortality due to pneumonia was 39.3 percent, the incidence being high in kids under sixth month of age. High percentage of pneumonic death was noted in October, November, April and May. Kaw and Khera (1970) in a survey study screened 1712 dead sheep belonging to four Government Sheep Breeding Farms in Jammu and Kashmir and found pneumonia in 689 (40.2%) cases.

A few more reports regarding the survey of the incidence of goat pneumonia based on the observations made from abattoirs and routine necropsies are available. Tiwari and Pandit (1964) examined 1385 goats and sheep at

four abattoirs at Madhya Pradesh and found that the incidence of pneumonia was 13 percent. Ramachandran and Sharma (1969) in another survey recorded that out of 1755 goats necropsied, 507 showed pneumonic lesions, the incidence being 28.22 percent. In the same study they also observed that the incidence of pneumonic lesions in goats tended to be higher than sheep and varied from 14 - 28 percent compared to 11 - 19 perdent in sheep.

## Aetiology of Pneumonia

Mckercher (1964) reported that, eventhough the aetiology of respiratory infections was quite numerous and complex, there was a general similarity of clinical picture in pneumonia. Pulmonary inflammation might be caused by inhaled irritant gases or aerosols, by aspirated foreign matter, by aerogenous or haematogenous viruses, bacteria, fungi, metazoa as well as microorganisms of uncertain classification (Jubb and Kennedy, 1970). In many instances the lesions were specific by virtue either of their anatomic pattern or the presende of causative agent in the lesion.

Many investigators have incriminated bacterial organisms as the causative agent of pneumonia, in sheep and

goats (Creche and Gochenour, 1936; Cambell et al. 1949; Hamdy et al. 1959; Bensal, 1967; Corrado, 1967; Sarkar and Bhattacharyya, 1975). The role of Pasteurella in the aetiology of pneumonia in goats and sheep had been reported by many workers (Marsh, 1953; Gourley andBarber, 1960; Mugera and Kramer, 1967; Stamp, 1968; Ramachandran and Sharma, 1969; Mohn and Utklev, 1974; Korikov, 1974). During the course of a detailed study of aetiological factors of pneumonia in cattle, Robert (1955) observed occurrence of Pasteurella in very many cases. Isolation of both P.multocida and P.haemolytica had also been recorded by Carter (1955, 1956); Collier et al.(1962) and Yamamoto et al. (1976)from the lung tissues of animals suffering from pneumonia. Isolation of P.multocida from the lung of pigs seriously affected with pneumonia had been reported by Sulochana and Abdulla (1970). Gourley and Barber (1960) reported the recovery of nine strains of P.haemolytica from naturally occurring cases of pneumonia and septicaemia in goats, in Uganda. Mugera and Kramer (1967) recovered P.haemolytica in an acute pneumonic outbreak in goats in Kenya. Working on the bacteriology of pneumonic lesions in sheep and goats in India, Bansal and Malik (1966) and Bansal (1967) made frequent isolation of P.multocida and P.haemolytica. Similar results were also obtained by Ramachandran and Sharma (1969) in their investigational studies on pneumonia. Mohn and Utklev (1974) and Sherif

et al. (1975) also recorded the incidence of pneumonia due to Pasteurella organisms in lambs.

Streptococcus as an aetiological agent of pneumonia in goats had been reported by several workers (Dhanda and Chandrasekariah, 1958; Corrado, 1967; Ramachandranand Sharma, 1969; Mazitov et al. 1973; Stevenson, 1974). In a comprehensive study of bacteriology of pneumonia in sheep and goats, Romer (1948) and Fey (1957) observed occurrence of large numbers of pathogenic strains of Str. pneumoniae in the lung tissues of affected animals. Experimental studies had revealed reproduction of typical lobar pneumonia in young as well as in adult animals. Wallman et al. (1955); Goldstein et al. (1967); and Blasilliere et al. (1968) also reported certain serotypes of Str. pneumoniae as the main pathogens associated with pneumonia, although other serotypes were also present in the nose and throats of healthy animals. In an outbreak of fatal respiratory infection in a flock of 100 sheep and 50 goats, Str. pneumoniae were isolated from the lungs of six dead animals (Corrado, 1967). Experimental studies had shown that the agent was pathogenic for mice and rabbits but not to guinea pigs.

The importance of corynebacterial organisms in pneumonic conditions in goats had been studied by some

workers (Gowan et al. 1957; Bansal and Malik, 1966; Natarajan and Nilakantan, 1974; Sarkar and Bhattacharyya, 1975). Ramachandran and Sharma (1969) reported that they were able to isolate C.pyogenes from 27 percent cases of caprine pneumonia. Isolation of C.ovis from the lungs of goats showing pneumonic lesions had been made by Sarkar and Bhattacharyya (1975). During the course of an investigational study of pathological conditions in sheep and goats in Nigeria, Addo (1976) made isolation of Corynebacterial organisms from 176 goats.

Contagious caprine pleuropneumonia caused by Mycoplasma mycoides var capri is one of the most serious enzootic diseasesof goats (Longley, 1951; Cordy et al.1955; Salisbury, 1957; Hudsen et al. 1967; Pearsen et al. 1972; St.George and Carmichael, 1975). Isolation of the Mycoplasma organism from caprine pleuropneumonia cases had been reported from many parts of the world (Babiker, 1968; Cottew et al. 1969; Perreau, 1971; Goni and Onoviran, 1973; Macowan and Minette, 1976). Reports on isolation of Mycoplasma from sheep pneumonia are also available from different parts of the world (Carmichael et al. 1972; Erdag, 1972; Sullivan et al. 1973; Alley and Mankteiow, 1975; St.George and Carmichael, 1975; Stipkovits et al. 1975).

Pneumonia in goats caused by Staphylococcus

streptococcus, klebstella pneumoniae and Escherichia coli had been reported by several workers (Bansal and Malik, 1966; Annonymus, 1967; Bansal, 1967, Austrian,1968; Ramachandran and Sharma,1969; Tanner et al. 1969; Stevensen, 1974; Smith and Williams, 1976).

## Pathogenicity Studies

The pathogenic properties of Pasteurella, for a wide variety of animals had been reported by several workers (Borgman and Wilson, 1955; Smith 1964; Biberstein et al. 1967; Bellavance et al. 1974; Lukyanenko et al. 1974; Thomas, 1974; Gilmer et al. 1975). Smith (1958) reported that the strains of P.multocida isolated from different sources varied in their virulence to mice. The author had substantiated that the strains of P.multocida isolated from cats were highly virulent when compared to the dog strain, which was moderately virulent. Mice, rabbits and pigeons were said to be the most suitable experimental animals for testing virulence of P.multocida (Wright, 1936). Bullen and Rogers (1968) reported that Pasteurella was able to produce the symptoms, even in passively immunised mice if intra-peritoneal injection of a solution of haemoglobin or ferric ammonium citrate was given. Intraperitoneal injection of a 24 hour broth suspension of P.multocida in rabbits resulted in death

after two or five days and the lesions noted were local oedema, congestion and in certain areas haemorrhagic tracheitis (Magnusson,1914). Sulochana and Abdulla (1970) also demonstrated pathogenicity of P.multocida isolated from pneumonic conditions in pigs, to mice, rabbits and to the primary host. Biberstein et al. (1967) conducted a study of the experimental production of pneumonia in sheep and the method of inoculation was by tracheo-bronchial intubation. Borgman and Wilson (1955) artificially produced pneumonia in goats with a 24 hour culture suspension of P.multocida by intratracheal route. However, mild febrile reaction was only noticed in cattle by intranasal instillation of P.multocida (Collier et al. 1960; Baldwine et al. 1967). But Gale and Smith (1958) reported that no reaction was noticed when similar organisms were injected subcutaneously although Collier (1968) could produce bronchopneumonia by the same procedure. Ramachandran and Sharma (1968) found that pneumonia could very well be produced by the haematogenous route in sheep and goats with agar embolic containing Pasteurella. Gilmer et al. (1975) also observed that experimental pneumonia could be produced with aerosol infection of P.haemolytica.

Several workers had reported the experimental pathogenicity and production of pneumonia in various

laboratory animals, using Streptococcus pneumoniae (Mazitov, 1973; Adams et al. 1974; Goldzinder, 1974). Str.pneumoniae was highly pathogenic when injected into mice and rabbits but guinea pigs were usually refractory; cat, dog and chicken are also said to be relatively resistant. In rabbits, it produced a fatal bacteraemia when given by intravenous route and an acute periotnitis followed by bacteraemia when injected intraperitoneally (Wilson and Miles, 1975). Some workers had reported that different serotypes varied in their virulence to different laboratory animals and also stated that the type of capsular polysaccharide was the determining factor for virulence (Schaffer, et al. 1936; Hjordis M.Foy, 1975). Dhanda and Chandrasekariah (1958) studied the pathogenicity of the strains of Str.pneumoniae isolated from pneumonic lesions of goats for mice. They found that 0.5 ml of $10^{-4}$ dilutions of 18 - 24 hour old serum broth culture was estimated to be the average lethal dose for mice. Corrado (1967) in an experimental study in mice, rabbit and guinea pig with a strain of Str.pneumoniae isolated from the pneumonic lung of goats reported that the agent was pathogenic for rabbit and mice and not for guinea pig. However, Adams et al. (1974) reported an outbreak of respiratory disease in guinea pigs accessible to Str. pneumoniae type XIX.

Corynebacterium pyogenes is a wide spread pathogen of domestic and experimental animals, and under natural conditions gave rise to suppurative pneumonia and other suppurative lesions (Rajagopalan, 1937; Magnusson, 1938; Roberts, 1957; Thal and Rutqvist, 1959). Subcutaneous injection of living culture of C.pyogenes into the rabbit brought about development of local abscess and if given by intravenous route resulted in generalization and abscess formation in bone and joints (Wilson and Miles, 1975). Brown and Orcutt (1920) reported that rabbit appeared to be most susceptible, guinea pigs was less susceptible and mouse was relatively resistant. Various authors had reported that under suitable conditions C.pyogenes liberated an exotoxin. This exotoxin was found to be lethal to mice and rabbits if injected intravenously and a dermonecrotic effect would be produced in guinea pigs.(Lovell, 1937; Soucck et al.1965; Smith, 1966). Arseculeratue and Navaratnane (1975) reported the isolation of C.bovis from the lung abscess of a laboratory rabbit and they experimentally reproduced a similar disease by intravenous injection of the isolate. Zaki (1976) reported the relationship of toxigenicity and pyogenicity in experimentally infected mice. They found that suspensions of C.ovis once subjected to physical and chemical treatment, would not be able to kill the mice, but was able to produce sterile pyogenic lesions.

## Antibiotic Susceptibility

Eventhough different types of invitro antibiotic tests have been used, the results were influenced sometimes markedly by the test conditions. The WHO Expert Committee (1961) classified the methods of invitro antibiotic sensitivity tests into two main categories: diffusion methods and dilution methods.

The principle of diffusion method is that the drug kept in a focus diffuses to the surrounding solid medium and causes a zone of inhibition of microorganisms present over the medium. The antibiotics are incorporated into the filter paper disc or in compressed tablet. The filter paper disc diffusion method had become the most commonly used and accepted procedure because of the simplicity, rapidity and availability of commercially prepared disc (Anderson, 1970; Garrod and O' Grady, 1971; Davis and Stout, 1971). In a survey, Castle and Elstub (1971) found that 99 percent of the laboratories in Britain used the disc diffusion test. The WHO Expert Committee (1961) had recommended standards of quality for commercial antibiotic sensitivity testing discs. Eriesson and Sherris (1971) and Garrod and Waterworth (1971) described varying methods in standardization since varying concentrations of disc along with different kinds of media

and techniques were employed by different workers. Bauer et al. (1966) advocated a standardised single, high potency disc method with the object that (1) the result obtained should be reproducible (2) Interpretation of results of the disc test was quantitative. By this method, the test organism could be classified into resistant/sensitive/ intermediate by referring to a zone interpretation chart. The above method is now known as Bouer-Kirby method and had been extensively evaluated and widely used (Matsen et al. 1970; Berry et al. 1970; Anderson, 1970). The Bauer-Kirby method is quantitative and is based on minimal inhibitory concentration (M.I.C.). Cheatle (1967) described the use of a zone size chart based on Bauer-Kirby method to simplify interpretation of zone diameters as a measure of bacterial response to an antibiotic. Oberhofer and Maddox (1970) described the use of an improved zone size chart which helps to eliminate the tedious task of measuring each zone. Patersdorf and Sherris (1965) standardized an interpretation chart to designate the organisms as resistant/sensitive/ intermediary which helped the workers for interpreting the result of they were using the high level disc diffusion method.

# MATERIALS AND METHODS

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## Isolation and Identification

Materials for this study were collected from the following sources:

1. The University Goat Farm, Mannuthy.
2. The Goat Unit of the All India Co-ordinated Research Project on Goat for Milk Production, Stationed at Mannuthy.
3. Municipal Slaughter House at Kuriachira, Trichur.

### Collection of Materials.

Nasal and laryngeal awabs were collected from 55 goats of different age groups manifesting clinical signs of pneumonia. Sterile cotton swabs moistened in sterile normal saline solution were used for obtaining materials from nostrils and laryngeal region.

Eighty specimens of lung tissues, 43 tracheal swabs and 36 samples of bronchial lymph nodes were collected aseptically from goats showing pneumonic lesions at the time of slaughter. The details of specimens are shown in Table 1.

Laboratory Procedure.

a) Swabs.

The swabs were directly inoculated on ten percent bovine blood agar, chocolate agar and serum agar and were incubated at 37°C for 24 to 48 hours under aerobic conditions. Another set of inoculated plates were incubated at 37°C under ten percent carbondioxide tension in order to obtain better growth of certain pathogens causing pneumonia. The swabs were also cultured on nutrient broth enriched with ten percent serum; and following 24 hours of incubation at 37°C, sub-cultures were made on blood agar plates and were further incubated under ten percent carbondioxide tension. Isolated colonies were picked up and streaked on fresh blood agar plate for further studies. The technique adopted for obtaining pure cultures was as advised by Cruickshank et al. (1975). The cultures isolated were identified by their morphological, cultural and biochemical characters as described by Cowan and Steel (1974).

b) Lung and Lymph nodes.

The surfaces of lung and lymph nodes were seared with hot spatula and opened by a sterile knife. Sterile cotton

swabs soaked in sterile buffered saline solution were thoroughly rubbed on the cut surface of the lesions. The swabs were then inoculated into the media mentioned above in order to obtain growth of organisms. Pure cultures obtained were transferred to blood agar slants and their identification was done in the same line as described above.

Twenty-five samples including nine nasal swabs and 16 lung tissues were used for isolation of Mycoplasma. For this, the lung samples along with the associated lymph nodes were pooled together and a 20 percent emulsion was made by grinding them with pestle and mortar. Nutrient broth was used as diluent. The emulsified tissues and nasal swabs were inoculated into tubes containing PPLO liquid medium as suggested by Chanock et al.(1962). Six culture tubes were used for each specimen examined. After seven days incubation at 37°C, two drops from each of the inoculated tube were streaked on PPLO agar plates as advocated by Arisoy et al. (1967). Incubated plates were examined under a stereoscopic microscope daily from second day for a period of two weeks.

## Pathogenicity Studies

Although several species of organisms were isolated during this investigation, only three species (P.multocida, Str.pneumoniae and C.pyogenes) were selected for conducting

pathogenicity studies.

1. Pasteurella multocida.

Out of 26 strains of Pasteurella multocida isolated during the course of the study, four (N140/76, L76/76, T43/76 and R80/76) were tested for their pathogenicity to laboratory experimental animals, mice and rabbits. A broth suspension of P.multocida washed from 24 hour old blood agar plates was used for the pathogenicity study. The broth suspension was incubated at 37°C for 18 hours and was diluted in sterile saline and the opacity was adjusted between five and six of Brown's opacity tube before experimental inoculation. In all instances suitable controls were maintained.

Mice.

Swiss albino suckling mice were injected subcutaneously with 0.1 ml broth suspension containing approximately $0.42 \times 10^7$ organisms. Two animals were used for each strain tested. The animals were observed for evidence of illness. Peripheral blood smears prepared from all the experimental animals at 24 hour intervals were examined to detect septicaemic stage of the infection. Heart blood and internal organs of six animals succumbed within 56 hours after experimental inoculation were cultured on blood agar, to detect bacterial

growth. Two animals which resisted infection were sacrificed on seventh day and tissues were subjected to similar studies.

Rabbits.

Subcutaneous injection of 0.5 ml of a suspension of P.multocida containing approximately 2.1 x $10^7$ organisms was made in eight rabbits of two months age. Blood smears prepared at 24 hour intervals were examined for the presence of organism. Tissues and fluids collected from animals that died at various intervals were processed and streaked on blood agar medium. The details of experiments are summarised in Table IIIb.

2. Streptococcus pneumoniae.

Out of 19 strains of Str.pneumoniae isolated from the pneumonic conditions, pathogenicity studies were conducted in experimental animals with four strains (N13/76, L67/76, T41/76,R66/76).

Mice.

Swiss albino mice aged one month were employed for this study. Two animals were used for each strain of Str. pneumoniae. The growth was emulsified in normal saline and

the opacity of the suspension was adjusted between five and six of the Brown's opacity tubes. 0.1 ml of the emulsion containing approximately 0.42 x $10^7$ organisms was given intraperitoneally to each animal. Peripheral blood smears were prepared and examined at 24 hour intervals. All the inoculated animals succumbed to infection within 72 hours of infection. Tissues and fluids from dead animals were subjected to detailed bacteriological studies.

Rabbits.

Eight healthy rabbits were inoculated with 0.5 ml of the culture emulsion containing approximately 2.1 x $10^7$ organisms intraperitoneally. Blood smears were examined at 24 hour intervals. Four animals died within 72 hours of inoculation and four resisted infection for 15 days till they were sacrificed. Tissues and fluids were examined in detail to detect bacterial infection. Details of the experiment are summarised in Table IIIb.

3. Corynebacterium pyogenes.

Out of 29 strains of C.pyogenes isolated, four strains (N28/76, L71/76, T39/76, R76/76) were utilised for experimental infection.

Mice.

Two animals were used for each strain tested. 0.1 ml of a broth suspension of the organism containing approximately $0.68 \times 10^7$ was injected intraperitoneally. Since the inoculated animals did not show any evidence of infection for a period of 15 days, they were sacrificed and their internal organs and fluids were examined for lesions and were processed for bacteriological examination.

Rabbits.

Intraperitoneal injection of 0.5 ml of a broth suspension of C.pyogenes containing approximately $3.4 \times 10^7$ organisms was given to eight rabbits in the same line described for mice. All the rabbits survived 15 days of inoculation and were sacrificed and autopsied. Tissues and fluids were processed for further studies. The details of experiments are illustrated in Table IIIb.

Pathogenicity Studies on Goats.

Since the various strains of the organisms isolated from pneumonic condition in goats were found to be pathogenic to some of the experimental hosts, their pathogenicity to the

primary host was also investigated.

Healthy goats, aged four to six months, were used for this study. The goats were divided into four groups, each group consisting of three animals. The animals were kept under close observation for one week before experimental infection. Thorough check up of the animals was done to eliminate the possibility of pre-existing pneumonia. Temperature was recorded daily for one week prior to the commencement of experiment. The fourth group was used as control.

The first group of three goats (432, 433 and 434) was inoculated with the strain (L76/76) of P.multocida isolated from a pneumonic lung. 1.5 ml of 1:10 dilution of an 18-hour old broth culture of P.multocida containing approximately $6.3 \times 10^7$ organisms was used as inoculum. Method of inoculation was by tracheobronchial intubation. Polyethylene tubes with smallopenings at the sealed end was passed into the trachea and presumably into the bronchi. The required quantity of the inoculum was poured into the tube through a funnel. In this way the inoculum was distributed in the form of a fine spray and had a better chance of being inhaled instead of being deposited in a mass at a particular spot in the lung.

Peripheral blood smears from inoculated and control animals prepared at 24 hour intervals were examined for bipolar

organisms. The temperature was recorded daily in the morning and evening throughout the experimental period. Animals destroyed on the 12th, 16th and 21st day of inoculation were autopsied and gross lesions were recorded. Impression smears from the lung, trachea, pericardium and heart blood were stained and examined. The heart blood and internal organs were subjected to detailed bacteriological studies. For histopathological studies, tissues were collected in ten percent neutral formalin and processed by the paraffin embedding method. Sections were stained by Hematoxylin and Eosin method. Details of the experiment are furnished in Table IV.

The second group of three goats (412, 414 and 417) was inoculated with the strain of Str.pneumoniae isolated from a pneumonic lung of goat (L67/76). 1.5 ml of a 1:10 dilution from 18 hour old broth culture of Str.pneumoniae containing approximately $6.3 \times 10^7$ organisms was used as inoculum. The method of inoculation was same as described above. Animals sacrificed at various intervals were examined in detail for evidence of infection. Heart blood and internal organs were cultured on blood agar under ten percent carbon-dioxide tension to detect bacterial growth. Histopathological studies were also conducted on formalin fixed tissues. The paraffin embedded sectionswere stained with H & E method.

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Details of the experiment are summarised in Table IV.

The third group of three goats (K38, K40 and K48) was inoculated with 1.5 ml of 1:10 dilution from 18 hour broth culture containing approximately 102 x $10^7$ organisms of C.pyogenes (L71/76) originally isolated from a pneumonic goat lung. Tissues and fluids from the goats sacrificed at 12th, 16th and 21st day after inoculation were screened for evidence of infection as described earlier.

One animal each from fourth group was also destroyed on 12th, 16th and 21st day and was examined in detail by cultural and histopathological studies.

## Antibiotic Sensitivity

Although several species of organisms were isolated during this investigation, ony 74 strains (26 P.multocida, 19 Str.pneumoniae and 29 C.pyogenes) were selected for conducting sensitivity studies.

The method employed for sensitivity testing was of high level disc-diffusion type. The following antimicrobial agents were employed for the study: ampicillin, bacitracin, chloramphenicol, erythromycin, gentamycin, kanamycin, nitrofurans, penicillin, streptomycin, tetracycline and triple sulfa*.

Disc preparation.

Discs of 6 mm diameter were punched from Whatman No.1 filter paper and were sterilized by dry heat at 140°C for one hour in lots of 100, in petri dishes. Standard suspensions of antibiotics were prepared in sterile distilled water. Dilutions of the antibiotics as shown in Table V were made according to the technique described by Blair et al. (1970). One drop of the dilution which will provide optimum concentration of antibiotic for the experimental purpose was absorbed in each disc. The antibiotic incorporated sensitivity discs were stored in sterile vials at 4°C until use.

Medium.

Nutrient agar enriched with five percent bovine defibrinated blood was used for growing the organisms for sensitivity studies.

Preparation of inoculum.

The strains to be tested were grown on blood agar medium for a period of 24 hours. Serum broth tubes were inoculated with five colonies removed from blood agar plates and incubated for five hours or until there was moderate

cloudiness equal to the standard described by Blair et al. (1970).

Inoculation of plates.

Blood agar plates were inoculated with the culture suspension by smearing the entire surface by cotton swab dipped in serum broth culture. The plates were then allowed to dry in the inverted position at room temperature for a period of five minutes.

Application of discs.

The discs containing different antibiotics mentioned above with appropriate concentration were placed on the medium suitably spaced and the plates were incubated at 37°C.

Reading of plates

The plates were read after overnight incubation. The diameter of the zone around each disc in which no growth was macroscopically discernible was measured with a pair of calipers, the diameter of disc also being included in the measurement. The findings were recorded and interpreted adopting the guide lines suggested by Patersdorf and Sherris (1965). (Appendix I).

# R E S U L T S

## RESULTS

Out of the 214 specimens examined, 139 (64.95%) revealed the presence of different types of organisms. No organisms could be isolated from 75 samples. Thirty-nine nasal swabs, 42 lung tissues, 30 tracheal swabs and 28 lymph nodes revealed evidence of bacterial growth (Table 1), the percentage being 70.9, 52.5, 69.8 and 77.8 respectively. One hundred and fifty-six strains of various bacterial species were isolated and identified, the important species being Pasteurella multocida, Streptococcus pneumoniae, Streptococcus pyogenes, Corynebacterium pyogenes, Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli (Table 1).

Out of 26 strains of P.multocida isolated, seven were from nasal swabs, five from the lung tissues, eight from tracheal swabs, and six from the bronchial lymph nodes. On blood agar, the majority of the isolates produced flat round translucent colonies with smooth surface on 24 hour incubation. However, a few of the isolates showed colonial dissociation turning to mucoid or rough character. No growth was observed on MacConkey's agar but luxurient growth was observed on KCN medium. No haemolysis was noted on bovine blood agar. Diffused cloudiness was noticed in broth following 24 hour incubation. Biochemically, catalase,

oxidase, acid from glucose, mannitol and sucrose were positive. Variable behaviour was noticed in arabinose, maltose, xylose, trehalose, sorbitol and also in ONPG, hydrogen sulphide and urease test. Ornithine decarboxylase, indole production and nitrate reduction were positive. Gelatin was not liquified. The results of detailed reactions of all the strains of P.multocida isolated are tabulated in Table IIa.

Among the 19 strains of Str.pneumoniae isolated during the course of this study, six were from nasal swabs, four from lung tissues, seven from the tracheal swabs and two from bronchial lymph nodes. Seven strains possessed capsule. Better growth was obtained in culture when 5 - 10 percent carbondioxide was provided for primary isolation. Colonies on blood agar were small and smooth with flat surface although a few strains showed rough and irregular colonies. Uniform turbidity in broth was produced by smooth colonies and granular growth by rough and irregular colonies. Alpha type of haemolysis was given by all the strains. Growth at 45°C, at pH 9.6, on ten percent bile and in 6.5 percent sodium chloride were negative. Catalase, oxidase, voges-proskauer, arginine hydrolysis, aesculin hydrolysis, gelatin liquefaction, hippurate hydrolysis and CAMP test were also negative. Acid clot on litmus milk was noticed. Bile solubility, optochin sensitivity, acid in glucose, lactose, maltose and sucrose

were positive. Glycerol, mannitol, salicin, sorbitol and arabinose were not fermented. Variable reactions were noticed on raffinose and trehalose. The results of the detailed reactions of all the strains of Str.pneumoniae are shown in Table IIb.

A total of 29 strains of C.pyogenes were isolated of which four strains were from nasal swabs, 11 from the lung tissues, six from the tracheal swabs and eight from the bronchial lymph nodes. Scanty growth was obtained on blood agar after 36 hours at 37°C. Growth was obtained both under aerobic and anaerobic incubation. Nitrate reduction, gelatin liquefaction, lactose, maltose, and haemolysin production were positive. Voges-Proskauer, hydrogen sulphide, urease and indole reaction were negative. Variable behaviour in mannitol and sucrose fermentation were noticed. The results of the detailed reactions of all the strains of C.pyogenes isolated are tabulated in Table IIc.

Among the 32 strains of Streptococcus pyogenes isolated during the course of this study, ten strains were from nasal swabs, nine from the lung tissues, five from tracheal swabs and eight from bronchial lymph nodes. The results of detailed reactions of all the strains of Str. pyogenes isolated are shown in Table IId.

A total of 33 strains of Staphylococcus were isolated of which 15 strains were from the nasal swabs, eight from the lung tissues, four from the tracheal swabs and the rest six from bronchial lymph nodes. The results of the detailed reactions shown by all the strains of Staph.aureus are tabulated in Table IIe.

Eleven strains of Klebsiella pneumoniae and six strains of E.coli were also isolated and studied. They were identified by the morphological, cultural and biochemical reactions as illustrated in Table IIf and IIg respectively.

Although 25 samples were processed for isolation of Mycoplasma species, none of them revealed presence of these organisms.

Pathogenicity Studies

Pasteurella multocida.

Mice.

All the eight animals inoculated with P.multocida cultures showed a rise of temperature varying from 2.5 - 3.5°F. Blood smears prepared from them at varying intervals showed

large numbers of bipolar stained organisms. Six animals succumbed to infection within 56 hours of inoculation. Gross lesions noticed in animals which died within 56 hours, were only local oedema and congestion at the site of inoculation. However, P.multocida was isolated from the heart blood and lung tissue of these animals. The remaining two animals sacrificed on seventh day showed fibrinopurulent pericarditis and partial consolidation of both lungs. In both cases P.multocida could be recovered from the heart blood, pericardial fluid and lungs. The result of experimental pathogenicity in mice are summarised in Table IIIa.

Rabbits.

Eight rabbits inoculated with P.multocida showed a rise in temperature of 3 to 4°F. Blood smears prepared from them showed presence of bipolars. All of them died within 56 hours. On autopsy, local oedema, congestion, haemorrhagic tracheitis and hyperaemia of kidney and intestine were noticed. In all these caass, smears and cultural examination revealed presence of organisms in heart blood, pericardial fluid and tracheal exudate. The results are summarised in Table IIIb.

Goats.

Goats (432, 433 and 434) inoculated with the strain

of P.multocida showed rise of temperature up to 105.5°F, from the second day onwards which persisted for five days. They had anorexia and slight mucopurulent nasal discharge at the end of the first week. However, blood collected during this period did not reveal any evidence of infection. The goat (432) destroyed on 12th day showed only slight tracheitis and a few consolidated areas in the lung (Fig.1). There was also slight oedema involving the cardiac and diaphragmatic lobes. Histologically the consolidated areas showed thickening of the alveolar walls with slight proliferation of septal cells. No frank exudation was seen within the alveoli. There was scanty cellular reaction in the mucosa of bronchi and bronchioles (Figs. 2 & 3). The epithelium of these structures was found intact. Peribronchial lymphoid accumulation was seen in many areas (Figs. 4 & 5). Tracheal swabs and lung cultures were positve for Pasteurella whereas heart blood cultures were negative.

The second goat (433) sacrificed on the 16th day of post inoculation revealed patchy areas of consolidation diffusely scattered in the left diaphragmatic lobe. At this stage also the alveolar septae were thickened and a few alveoli had some cell debris. The septal cell had proliferated and few of them were found free in the alveolar lumen. There was massive peribronchiolar lymphoid hyperplasia

in the form of nodules. Active germinal centres were observed. The pericardial fluid and heart blood were negative whereas the lung cultures revealed P.multocida.

The third goat (434) autopsied on the 21st day showed few focal consolidated areas on the cardiac lobe. The characteristic feature of the lung lesion at this stage was the presence of numerous large macrophages in the alveolar wall and in the lumen. The lymphoid nodule in the peribronchial and bronchiolar area showed tendency for encapsulation. The lung culture was positive. The results of experimental pathogenicity are summarised in Table IV.

Streptococcus pneumoniae.

Mice.

All the mice inoculated with strains of Streptococcus pneumoniae culture showed temperature reaction following twenty-four hour of inoculation and died within a period of seventy-two hours. The only predominant lesion noticed on necropsy was acute peritonitis. Isolations were made from heart blood, peritoneal fluid and lung. Details of the results are shown in Table IIIa.

Rabbits.

All the rabbits showed a rise of temperature of 2 to 3°F; however, blood smears did not reveal any organism. Four of them died within 72 hours. The animals which survived did not show any thermal reaction 72 hours after inoculation. Necropsy of the dead animals showed peritonitis with accumulation of fluid in the cavity along with spreading inflammatory lesions. Four animals sacrificed on 15th day postinoculation did not reveal any gross lesions. Heart blood and lung of the dead animals revealed presence of organisms whereas the sacrificed group did not show any bacterial growth on cultural examination. The details of the result are shown in Table IIIb.

Goats.

The second group of three goats (412, 414 and 417) inoculated with Str.pneumoniae showed a temperature reaction of 3.5°F after 36 hours of inoculation which persisted for a week. Blood films and cultural examinations were negative. The first goat (412) destroyed on the 12th day of inoculation had oedema and focal consolidation in the apical, cardiac and diaphragmatic lobes (Fig. 6). Histologically there was slight congestion, oedema and scanty cellular reaction. The bronchial and bronchiolar walls showed moderate infiltration with mononuclear cells (Figs. 7 & 8). Peribronchially there was diffuse

lymphoid reaction. Impression smears and lung culture showed the presence of organism, typical of the strain inoculated.

The second goat (414) sacrificed on the 16th day of postinoculation showed focal consolidated areas on the cardiac and diaphragmatic lobe of the right and left side. Consolidated areas showed infiltration with macrophages and few lymphoid cells. The peribronchial lymphoid reaction persisted and in addition a scanty cellular infiltration in the mucosa of some bronchioles was seen. Cultures and smears of lung showed presence of the organisms.

The third goat (417) autopsied on the 21st day after inoculation also revealed slightly firm focal areas on the right intermediate lobe and left apical lobe. The alveoli were completely free of any cellular reaction. The lymphoid reaction around the bronchi and bronchioles was still a constant feature. Lung cultures revealed presence of the organisms but the smears were negative. Details of the results are shown in Table IV.

Corynebacterium pyogenes.

Mice.

Eight mice inoculated with four strains of C.pyogenes,

survived even after 14 days without any adverse effects. Autopsy of these animals on 15th day showed presence of white nodules in the omentum and liver of only four animals. Organisms were isolated from the animals that showed lesions. No lesions were seen and no isolations could be made from the rest.

Rabbit.

Of the eight rabbits inoculated with C.pyogenes, four showed a temperature reaction of 2.5°F. although the blood cultures were negative. When destroyed and examined on the 15th day postinoculation, four rabbits showed localised nodules and suppurative lesions on the omentum and liver. C.pyogenes could be recovered from these lesions. The other four rabbits did not show any evidence of infection on postmortem examination.

Goats.

The third group of goats (K38, K40 and K48) inoculated with C.pyogenes had a temperature rise of 1.5 to 2°F. after 48 hours and then gradually came to normal within four days. All the three had slight nasal discharge which became muco-purulent later. The first goat (K38) autopsied on the 12th day showed focal pneumonic lesions in all the lobes(Fig. 9).

The alveolar wall in many areas had undergone necrosis. Most of the alveoli contained exudate consisting mainly of cellular debris admixed with large intact mononuclear phagocytes and few neutrophils (Fig. 10). Some of these macrophages were found in close proximity and in contact with the alveolar septae. The bronchi and bronchiolar epithelium had undergone moderate hyperplasia and the lumen contained exudate (Fig. 11). Peribronchially there was lymphoid reaction which had active germinal centres (Fig. 12). In addition to the pale blastoid centre in this nodules, a few plasma cells were also found at the periphery. The lung and tracheal swab culture revealed the presence of organisms.

The second goat (K40) autopsied on the 16th day had consolidation of the posterior and anterior part of the cardiac lobe on the right side; posterioventral aspect of the apical, entire cardiac and anterodorsal aspect of the diaphragmatic lobe of the left side also revealed pneumonic changes. A narrow elongated strip of consolidation was noticed on the anteroventral aspect of the diaphragmatic lobe. At this stage necrotic areas were seen scattered in the apical part of the lung. Many of the intact alveoli had plaques of necrotic mass. Large macrophages were seen both in the lumen and in the septae. The epithelium of the bronchi and bronchioles showed slight hyperplasia. The lymph-nodules were still present.

Lung culture revealed the presence of organisms.

The goat (K48) necropsied on the 21st day, the lesionswere almost similar to as on the 16th day, but slightly less in extent. Histologically the cellular reaction was of the macrophage type eventhough a few neutrophils were also seen (Fig. 13). Detailed results are shown in Table IV.

The fourth group of three goats (808, 422 and 427) destroyed and examined on 12th, 16th and 21st day did not show any evidence of infection either by cultural or histopathological examination.

## Antibiotic Sensitivity

Out of the 156 strains isolated during this study 74 strains (26 strains of P.multocida, 19 strains of Str. pneumoniae and 29 strains of C.pyogenes) were tested for the antibiotic sensitivity and the results of the tests are tabulated in Table V.

### P.multocida.

Sensitivity studies on 26 strains of P.multocida to 11 chemotherapeutic agents have shown the sensitivity

pattern as follows:

Ampicillin, 26.92%; bacitracin, 65.38%; chloramphenicol, 92.3%; erythromycin, 53.8%; gentamycin, 57.69%; kanamycin, 46.15%; nitrofurans, 100%; penicillin, 53.84%; streptomycin, 53.84%; tetracycline, 84.61%; and triple sulfa, 6.53%.

Str.pneumoniae.

Sensitivity of 19 strains of Str.pneumoniae to 11 chemotherapeutic agents was as detailed below:

Ampicillin, 84.21%; bacitracin, 94.75%; chloramphenicol, 100%; erythromycin, 84.21%; gentamycin, 36.84%; kanamycin, 42.10%; nitrofurans, 89.47%; streptomycin, 15.78%; penicillin, 100%; tetracycline, 31.57% and triple sulfa, 5.26%

C.pyogenes.

The result of 29 strains of C.pyogenes tested against the same agents was as follows:

Ampicillin, 100%; bacitracin, 10.34%; chloramphenicol 100%; erythromycin, 79.31%; gentamycin, 72.41%;

kanamycin, 72.41%; nitrofurans, 89.65%; penicillin, 100% and tetracycline, 51.72%. All the 29 strains were found to be resistant to streptomycin andto triple sulfa.

# DISCUSSION

## DISCUSSION

A number of workers have reported the association of P.multocida, Str.pneumoniae, C.pyogenes, Str.pyogenes, Staph. aureus, K.pneumoniae and E. coli, in pneumonic conditions in goats (Borgman and Wilson, 1955; Bansal, 1967; Corrado, 1967; Sarkar and Bhattacharyya, 1975). In the present study 26 strains of P.multocida, 19 strains of Str.pneumoniae, 29 strains of C.pyogenes, 32 strains of Str.pyogenes, 33 strains of Staph. aurues, 11 strains of K.pneumoniae and 6 strains of E.coli were isolated and identified.

P.multocida is generally accepted as the aetiological agent of pneumonia, when it can be isolated from the infected tissues (Gale and Smith, 1958; Gourlay and Barber, 1960). Even if the organism is not the sole cause, it is evidently of importance in producing in part, the characteristic pathological changes. It may be the normal inhabitant of the nasal mucosa and thus it is impossible to eliminate it from the animal colony (Carter, 1955). Yet studies in sheep have failed to reveal the organism in normal lung tissue (Borgman and Wilson, 1955). However, all the animals from which P.multocida was isolated in this study had clear evidence of respiratory disease manifested by laboured breathing and nasal discharge. Pneumonia usually does not develop unless there are predisposing

causes which include seasonal changes and stress factors (Kirton et al. 1976).

The colonial variation of P.multocida was observed on a few occasion. Those strains which showed colonial dissociation to rough character might be those belonging to intermediary group and one which showed mucoid character might be belonging to the flourescent group, as stated by Carter (1958). The strains studied also showed great variation in their ability to ferment carbohydrates (Table IIa). It is generally agreed that P.multocida ferments sucrose and mannitol but depicts variable behaviour in arabinose, sorbitol, lactose, maltose, xylose and trehalose (Fitch and Nelsen, 1923). Eventhough there was variability in the biochemical reaction, it is very difficult to assess the biotype based on these reactions alone. Khalifa (1934) and Robert (1947) classified the biotype of P.multocida based on the differences in the fermentation of mannitol, arabinose and xylose. But earlier Tanaka (1926) had reported that it was very difficult to assess the biotype based on the differences, in sugar fermentation alone.

The role of Str.pneumoniae in the induction of pneumonia had been reported by many workers (Dhanda and Chandrasekariah, 1958; Corrado, 1967, Mazitov, et al. 1973). A total of 19 strains of Str.pneumoniae was isolated during this

investigation. Animals from which isolations were made had apparent symptoms of pneumonia. The percentage of incidence was 12.8, of the total number of specimens examined. Ramachandran and Sharma (1969) in a detailed survey of the incidence of pneumonia in goats reported the occurrence of Str. pneumoniae in 10.8% of cases examined by them. Normally organisms grow in artificial media as raised circular colonies with smooth surface. Most of the strains agreed with this common pattern of colonial growth on the solid media used during this study. However, a few strains developed rough and irregular characters exhibiting colonial dissociation. Similar observation in colonial variation of Str.pneumoniae had also been made by Austrian (1953) in his studies on characters of Str.pneumoniae. With regard to the biochemical reactions all the 19 isolates showed a uniformity in their action except on raffinose and trehalose. All the isolates were soluble in bile and were sensitive to 5 mcg of optochin. Bowers and Jeffries (1955) reported that bile solubility and optochin sensitivity are the two most reliable methods of identifying Str.pneumoniae from other Streptococcus, eventhough Lund (1959) stated that optochin sensitivity was the most reliable method than bile solubility test. It is very difficult to assess the biotype of isolates with the biochemical reactions alone (Lund, 1970).

In the present study, 29 strains of C.pyogenes were obtained from materials collected from animals showing clinical signs of pneumonia, the incidence being 18.59%. Association of C.pyogenes in pneumonic conditions in goats had already been established by previous workers in their studies on respiratory diseases in goats (Bansal and Malik, 1966; Bansal, 1967; Sarkar and Bhattacharyya,1975). The isolates showed minute dew drop like colonies with a zone of haemolysis on blood agar as stated by Hugh and Leifson (1953) and Hermann, (1961). Marked variations were noted in their ability to ferment sugars like salicin, sucrose, xylose and mannitol, eventhough lactose and maltose were fermented uniformly by all these strains. Significant differences in their ability to ferment sugars had also been observed by other workers in their investigational studies on C.pyogenes (Natarajan and Nilakanta, 1974).

Out of the four strains of P.multocida inoculated into mice, three strains proved to be lethal to mice producing death in 56 hour. It is interesting to note that the above three strains of P.multocida which produced death in 56 hours exhibited smooth colonies when grown on solid medium. The lesions produced by these strains were not very marked except local oedema and congestion at the site of inoculation. The reason for the absence of typical lesions of P.multocida might be due to the acute course of the disease. On the other hand

two animals sacrificed on the seventh day had developed typical lesions of fibrinopurulent pericarditis and consolidation of lung although the strain inoculated in these animals was of rough variety. Rough colonies are less virulent to mice when compared to smooth type (Carter, 1958). But they are able to produce pathological changes in the tissues eventhough affected animals could survive for longer period of time. Rabbits inoculated with P.multocida succumbed to infection within 56 hours of inoculation manifesting minor pathological changes like local oedema, congestion and haemorrhagic tracheitis. Similar observations were also made by Magnusson (1914) in his experimental infection studies on P.multocida in rabbits. But Smith (1958) generalised that organisms producing rough colonies were less pathogenic to rabbits when compared to smooth type. However, no such variations were noted with regards to the degree of virulance among the species of organism used in the present study.

Goats inoculated with P.multocida showed marked therma reaction from second day onwards which persisted for five days. They had anorexia and slight mucopurulent nasal discharge at the end of first week. However, blood smears examined at various stages did not show any evidence of infection. The goat destroy on twelth day postinoculation had only slight tracheitis and consolidation of lungs. Histopathological examinations also

did not reveal any extensive changes except proliferation of septal cells and slight cellular reactions in the bronchi and bronchioles. However, P.multocida could be isolated from lung tissues of all the three experimental animals. The mild pneumonic changes noticed in these animals need not be considered as an active phase of a progressive infection caused by the organism. The experimental animals were maintained under ideal conditions throughout the period of the experiment and they were given good nourishing food. Borgman and Wilson(1955) and Dungworth and Cordy (1962) in their studies on pneumonia due to Pasteurella organism in goats and sheep, reported the influence of stress in production of pneumonia. They stated that lowered resistance by stress factors played an important role in causing pneumonia rather than infectious agent alone.

All the mice which were inoculated intraperitoneally with Str.pneumoniae succumbed to infection within 72 hours postinoculation indicating that these strains were lethal to mice. The lesions noticed were acute peritonitis and slight congestion of lung. Organisms were reisolated from tissues. Similar results were observed by Dhanda and Chandrasekariah (1958) and Adams et al. (1974). Two strains of Str.pneumoniae were pathogenic for rabbits killing the animals in 72 hours whereas the animals inoculated with other two strains remained healthy until they were destroyed on 15th day. On postmortem

examination the internal organs of these two group of animals also did not show any lesions whereas the animals died within 72 hours had acute peritonitis, suppurative changes and spreading inflammatory reactions. This variation is pathogenicity might probably be due to the lack of capsular polysaccharide in some, since capsular polysaccharide is the determining factor for virulence (Schaffer et al. 1936; Hjordis, 1975). It has also been observed that different serotypes varied in their virulence to different laboratory animals.

The goats inoculated with Str.pneumoniae showed temperature reaction for a week after which the animals became normal until they were sacrificed at varying intervals. Pathological changes were more pronounced in the animal sacrificed on 12th day. However, in goats sacrificed on 16th and 21st days these changes seem to be in the healing stage revealing that Str.pneumoniae has only a limited role in causing progressive pneumonia in goats when the animals are kept under ideal conditions. Although lesions were not very marked, lung cultures proved to be positive in all three cases. However, Dhanda and Chandrasekariah (1958) in a similar study in goats with Str.pneumoniae successfully reproduced fatal septicaemia by subcutaneous infection of broth culture. Survival of the organism in lung tissues even after apparent recovery from infection denotes that the disease could flare

up at a later time when the resistance of the host is being lowered due to some reason or other.

Among the mice inoculated with C.pyogenes only four showed nodules in the omentum and liver and the organism could be isolated from these lesions. Other four animals did not show any sign of infection when destroyed on 15th day of experiment. Moreover, no lesions could be detected in any animals in the respiratory tract. These findings show that the strains were not capable of inducing pneumonia although they were localised in omentum and liver. Experimental pneumonia could not be produced in rabbits although suppurative lesions were observed in omentum and liver. It has been reported that C.pyogenes as a wide spread pathogen of domestic and experimental animals and under natural condition, gave rise to suppurative pneumonia and other suppurative lesions (Rajagopalan, 1937; Magnusson, 1938; Roberts, 1957; Thal and Rutquist, 1959).

Moderate lesions of pneumonia were observed in goats sacrificed on 12th and 16th day of infection characterised by presence of exudate and cell debris in the alveoli along with infiltration of mononuclear phagocytes and hyperplasia of bronchial epithelium. However, the above lesions were not so pronounced in animal sacrificed on 21st day indicating

a steady recovery from infection. Moreover, isolation attempts also proved to be negative in animal sacrificed on 21st day. C.pyogenes has produced not only inflammatory cellur exudate, but also necrosis of pulmonary tissue in many locations. Compared to the other pathogenic organisms investigated it could be said that C.pyogenes had produced more intense tissue reaction in the lungs under identical conditions. All the strains studied produced lymphoid nodule formation in pulmonary tissue of goats with the formation of germinal centres. This indicated an immunological reaction.

Invitro antibiotics test provide a useful tool for assessing the possible effectiveness of antibiotics against infection. Eventhough there were several reports on the effect of drugs on bovine strains of P.multocida (McNeil and Hinshaw, 1948; Muysson and Carter, 1958; Karlsson and Mystrom, 1962; Fox et al. 1971; Khalyapina et al. 1972) similar accounts relating to goats are few. The study on the antibiotic sensitivity of the 26 strains of P.multocida showed that all the strains were sensitive to nitrofurans. Similar observations were made by Hariharan (1972) in the studies on P.multocida isolated mostly from canine and feline species. The next drug of choice was found to be chloramphenicol (92.3%). Tetracycline also showed a high percentage of sensitivity of 84.61. High percentage of sensitivity of P.multocida isolated from pigs

to tetracycline and chloramphenicol was observed by Abdulla and Sulochana (1971) in their studies on the sensitivity pattern of the strain to various chemotherapeutic agents. Eventhough drug resistance to chloramphenicol against the gram negative bacteria has been reported (Brock 1964) no such resistance pattern could be observed in this study. In the present study 46.16% of the strains tested were found to be resistant to penicillin. On the other hand Hariharan (1972) and Mackry et al. (1965) observed a penicillin resistance of 18.9% and 33.8 - 38.9%. Ampicillin and Kanamycin were found to be least effective against P.multocida the percentage being 26.92 and 46.15 respectively.

Antibiotic sensitivity studies in 19 strains of Str.pneumoniae have revealed a cent percent sensitivity to penicillin and chloramphenicol., 94.73% to bacitracin., 89.47% to nitrofurans., 84.21% to erythromycin and ampicillin indicating that the drug of choice after penicillin and chloramphenico being bacitracin, nitrofuran, erythromycin and ampicillin, High percentage of sensitivity of pneumococci to penicillin had also been observed by Zadovornyak and Bronschtein (1969) in their studies on pneumococci in infants. The percentage of sensitivity to kanamycin,gentamycin and tetracycline was moderately low being 42, 36.84 and 31.57 respectively.

Perival et al. (1969) reported that 12% of pneumococci isolated from respiratory diseases was resistant to tetracycline whereas Zadovornyak and Bronshtein (1969) found 50% pesistant strains to the same drug. Streptomycin (15.78%) and triple sulfa (5.26%) were found to be least active against Str.pneumoniae.

The sensitivity pattern of C.pyogenes to 11 antibiotics indicate cent percent sensitivity to ampicillin, chloramphenicol and penicillin. The drug sensitivity studies by Louisweinstein (1965) also revealed that penicillin and chloramphenicol are the two drugs which have the maximum invitro drug sensitivity against Corynebacterium. Nitrofurans and erythromycin are the next drugs of choice since they also had sensitivity of 89.65 and 79.31% respectively. Tetracycline showed 51.72% of sensitivity and all the strains were resistant to streptomycin and triple sulfa. Gentamycin and kanamycin also showed fairly high percentage of sensitivity of 72.41. Corynebacterial isolates completely resistant to streptomycin had been reported by Hariharan (1972).

An antibiotic resisttance occurs if the susceptibility spectrum of the organism becomes narrower increasing the complexity of treatment. If the trend is allowed to progress unchecked, increase of resistant population of bacteria could outrun the development of new therapeutic antibiotics (Smith 1969; Finland, 1970).

# S U M M A R Y

## SUMMARY

A total of 214 specimens from clinical cases and from slaughtered goats showing lesions of pneumonia was examined. Out of 214 specimens examined, 139 (64.95%) revealed the presence of different species of organisms. No organisms could be isolated from 75 samples. One-hundred and fifty six strains of bacterial species were isolated. Characterisation and classification of bacterial species were done based on their morphological, cultural and biochemical behaviour. The bacteria included 26 strains of P.multocida, 19 strains of Str.pneumoniae, 32 strains of Str.pyogenes, 29 strains of C.pyogenes, 33 strains of Staph.aureus,11 strains of K.pneumoniae and 6 strains of E.coli. The enrichment and selective media used for isolation included Blood agar, Chocolate agar,Serum agar, MacConkey's agar and KCN medium.

Although several species of organisms were isolated during this investigation three species (P.multocida,Str. pneumoniae and C.pyogenes)were used for conducting the pathogenicity studies in mice and rabbits as well as in the primary host. P.multocida and Str.pneumoniae were found to be lethal to laboratory animals in experimental inoculation. Laboratory animals inoculated with C.pyogenes showed suppurative changes in liver and omentum when sacrificed on the 15th day

following experimental transmission of the organisms.

Pathogenicity studies on primary host with P.multocida revealed thermal reaction and mild pneumonic changes characterised by few consolidated areas in lung, thickening the alveolar walls and infiltration of macrophages. However, these lesions were found to be resolving when examined on 21st day following infection.

Animals injected with Str.pneumoniae had developed pneumonic changes when destroyed and examined on the 12th, 16th and 21st day of inoculation. Infiltration of macrophages and lymphoid cells were also noticed.

Goats inoculated with C.pyogenes showed marked necrosis of alveolar walls and exudates containing mainly of cell debris admixed with large intact mononuclear phagocytes. Moreover, the epithelium of bronchi and bronchioles have undergone moderate hyperplastic changes.

Compared to P.multocida and Str.pneumoniae it could be stated that C.pyogenes have produced more intense tissure reaction in the lungs under identical conditions. The inoculated organisms could be recovered from the lung tissues of goats when destroyed at various intervals.

Eventhough the extent and nature of the pneumonic lesions varied one constant feature seen in all cases was lymphoid infiltration in the form of nodules which were seen peri-bronchially. Many of these nodules had active germinal centres. Such a reaction was considered to be due to an immunological reaction.

Invitro antibiotic sensitivity studies using ampicillin, bacitracin, chloramphenicol, erythromycin, gentamycin, kanamycin, nitrofurans, penicillin, streptomycin, tetracycline and triple sulfa were carried out in order to assess the effectiveness of these drugs in cases of pneumonia caused by these species. The drugs of choice for P.multocida were found to be nitrofurans (100%), chloramphenicol (92.3%) and tetracycline (84.6%). In thecase of Str.pneumoniae, penicillin (100%), chloramphenicol (100%) and bacitracin(94.73 %) were found to be effective and for C.pyogenes, ampicillin, chloramphenicol and penicillin showed cent percent sensitivity.

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# APPENDIX

Appendix I. Zone size interpretive chart used for disc sensitivity test.

| Antibiotic or chemotherapeutic agent | Disc potency | Inhibition zone. Diameter in mm. | | |
|---|---|---|---|---|
| | | Resistant | Intermediate | Sensitive |
| Ampicillin | 10 mcg | 20 or less | 21 - 28 | 29 or more |
| Bacitracin | 10 ,, | 8 ,, | 9 - 12 | 13 ,, |
| Chloramphenicol | 30 ,, | 12 ,, | 13 - 17 | 18 ,, |
| Erythromycin | 15 ,, | 13 ,, | 14 - 17 | 18 ,, |
| Gentamycin | 10 ,, | -- | -- | 13 ,, |
| Kanamycin | 30 ,, | 13 or less | 14 - 17 | 18 ,, |
| Nitrofurans | 300 ,, | 8 ,, | 9 - 12 | 13 ,, |
| Penicillin | 10 units | 20 ,, | 21 - 28 | 29 ,, |
| Streptomycin | 10 mcg | 11 ,, | 12 - 14 | 15 ,, |
| Sulfonamides | 300 ,, | 12 ,, | 13 - 16 | 17 ,, |
| Tetracycline | 30 ,, | 14 ,, | 15 - 18 | 19 ,, |

# T A B L E S

Table 1. Details showing the number of specimens examined and the species of Organisms isolated

| Type of Specimens | No.of specimens examined | No.of positive specimens | No.of negative specimens | P. multocida | Str.pneumoniae | C. pyogenes | Str.pyogenes | Staph. aureus | K.pneumoniae | E. coli |
|---|---|---|---|---|---|---|---|---|---|---|
| Nasal swabs | 55 | 39 | 16 | 7 | 6 | 4 | 10 | 15 | 2 | 1 |
| Lung pieces | 80 | 42 | 38 | 5 | 4 | 11 | 9 | 8 | 3 | 2 |
| Tracheal swabs | 43 | 30 | 13 | 8 | 7 | 6 | 5 | 4 | 6 | 0 |
| Bronchial lymph node | 36 | 28 | 8 | 6 | 2 | 8 | 8 | 6 | 0 | 3 |
| Total ... | 214 | 139 | 75 | 26 | 19 | 29 | 32 | 33 | 11 | 6 |

Table IIa. Detailed reactions of cultures of *Pasteurella multocida* isolated from pneumonic goats.

| Culture Numbers | N111/75 | N914/75 | N50/76 | N660/76 | N637/76 | N707/76 | N140/76 | L2/75 | L24/75 | L29/75 | L51/76 | L76/76 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Shape | R | R | R | R | R | R | R | R | R | R | R | R |
| Motility | - | - | - | - | - | - | - | - | - | - | - | - |
| Growth in Air | + | + | + | + | + | + | + | + | + | + | + | + |
| Growth Anaerobically | + | + | + | + | + | + | + | + | + | + | + | + |
| Catalase | + | + | + | + | + | + | + | + | + | + | + | + |
| Oxidase | + | + | + | + | + | + | + | + | + | + | + | + |
| Glucose (Acid) | + | + | + | + | + | + | + | + | + | + | + | + |
| Carbohydrates (F/O) | F | F | F | F | F | F | F | F | F | F | F | F |
| Growth on Mac Conkey's Agar | - | - | - | - | - | - | - | - | - | - | - | - |
| Growth on KCN | + | + | + | + | + | + | + | + | + | + | + | + |
| Arabinose | - | - | + | - | - | + | + | - | - | + | - | + |
| Lactose | - | - | - | + | - | - | - | - | - | + | - | - |
| Maltose | - | + | - | + | - | - | + | - | - | - | - | + |
| Mannitol | + | + | + | + | + | + | + | + | + | + | + | + |
| Raffinose | - | - | - | - | - | - | - | - | - | - | - | - |
| Salicin | - | - | - | - | - | - | - | - | - | - | - | - |
| Sorbitol | + | - | + | - | + | + | - | + | + | - | + | + |
| Sucrose | + | + | + | + | + | + | + | + | + | + | + | + |
| Trehalose | - | - | + | - | - | + | - | - | + | - | - | - |
| Xylose | + | - | - | - | - | - | - | - | - | + | - | - |
| Aesculin hydrolysis | - | - | - | - | - | - | - | - | - | - | - | - |
| Urease | - | - | - | - | - | - | + | - | - | - | - | + |
| Hydrogen sulphide | - | - | + | - | + | - | + | - | - | - | - | + |
| Ornithine decarboxylase | + | + | + | + | + | + | + | + | + | + | + | + |
| Indole | + | + | + | + | + | + | + | + | + | + | + | + |
| ONPG | - | - | - | + | - | - | - | + | - | + | + | - |
| Gelatin hydrolysis | - | - | - | - | - | - | - | - | - | - | - | - |
| Nitrate reduction | + | + | + | + | + | + | + | + | + | + | + | + |
| Haemolysis | - | - | - | - | - | - | - | - | - | - | - | - |

Table IIa (Contd.). Detailed reactions of cultures of Pasteurella multocida isolated from pneumonic goats.

| Culture Numbers | T1/75 | T7/75 | T14/75 | T20/75 | T29/75 | T34/76 | T40/76 | T43/76 | R44/76 | R51/76 | R57/76 | R62/76 | R71/76 | R80/76 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Shape | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| Motility | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Growth in Air | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Growth Anaerobically | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Catalase | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Oxidase | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Glucose (Acid) | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Carbohydrates | F | F | F | F | F | F | F | F | F | F | F | F | F | F |
| Growth on Mac Conkey's | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Growth on KCN | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Arabinose | - | - | - | - | - | - | - | + | - | - | - | + | - | - |
| Lactose | - | - | - | - | - | - | - | - | - | + | - | - | + | - |
| Maltose | - | - | - | - | - | - | - | + | - | - | - | + | - | - |
| Manitol | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Raffinose | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Salicin | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Sorbitol | + | + | - | + | - | + | + | + | + | + | - | + | + | + |
| Sucrose | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Trehalose | + | - | - | - | + | - | - | + | - | + | - | - | - | - |
| Xylose | - | - | + | - | - | - | - | + | - | - | + | + | - | + |
| Aesculin hydrolysis | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Urease | - | - | - | + | - | - | + | - | - | + | - | - | + | + |
| Hydrogen sulphide | - | + | - | - | + | - | - | - | - | - | + | - | - | + |
| Ornithine decarboxylase | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Indole | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| ONPG | - | - | - | - | - | + | - | - | - | + | - | - | + | - |
| Gelatin hydrolysis | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Nitrate reduction | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Haemolysis | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

R = Rod-shaped; F = Fermentative; + = Positive; - = Negative

Table IIb. Detailed reactions of cultures of *Streptococcus pneumoniae* from pneumonic goats

| Culture Numbers | N2/75 | N142/75 | N108/75 | N244/75 | N015/76 | N13/76 | L21/75 | L33/76 | L52/76 | L67/76 |
|---|---|---|---|---|---|---|---|---|---|---|
| Shape | S | S | S | S | S | S | S | S | S | S |
| Acid fast | - | - | - | - | - | - | - | - | - | - |
| Spores | - | - | - | - | - | - | - | - | - | - |
| Motility | - | - | - | - | - | - | - | - | - | - |
| Growth in Air | + | + | + | + | + | + | + | + | + | + |
| Catalase | - | - | - | - | - | - | - | - | - | - |
| Oxidase | - | - | - | - | - | - | - | - | - | - |
| Glucose | + | + | + | + | + | + | + | + | + | + |
| OF | F | F | F | F | F | F | F | F | F | F |
| Haemolysis | a | a | a | a | a | a | a | a | a | a |
| Growth at 45°C | - | - | - | - | - | - | - | - | - | - |
| Growth at pH 9.6 | - | - | - | - | - | - | - | - | - | - |
| Growth in 6.5% sodium chloride | - | - | - | - | - | - | - | - | - | - |
| Growth on 10% bile | - | - | - | - | - | - | - | - | - | - |
| V.P. | - | - | - | - | - | - | - | - | - | - |
| Arginine hydrolysis | - | - | - | - | - | - | - | - | - | - |
| Aesculin hydrolysis | - | - | - | - | - | - | - | - | - | - |
| Litmus milk | AC | AC | AC | AC | AC | AC | AC | AC | AC | AC |
| Gelatin liquefaction | - | - | - | - | - | - | - | - | - | - |
| Hippurate hydrolysis | - | - | - | - | - | - | - | - | - | - |
| CAMP test | - | - | - | - | - | - | - | - | - | - |
| Bile solubility | + | + | + | + | + | + | + | + | + | + |
| Optochin sesnsitivity | + | + | + | + | + | + | + | + | + | + |
| Glycerol | - | - | - | - | - | - | - | - | - | - |
| Lactose | + | + | + | + | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + | + | + | + | + |
| Mannitol | - | - | - | - | - | - | - | - | - | - |
| Raffinose | - | + | - | - | + | - | - | + | + | + |
| Salicin | - | - | - | - | - | - | - | - | - | - |
| Sorbitol | - | - | - | - | - | - | - | - | - | - |
| Sucrose | + | + | + | + | + | + | + | + | + | + |
| Trehalose | + | - | + | - | + | + | - | - | - | + |
| Arabinose | - | - | - | - | - | - | - | - | - | - |

Table IIb. (Cont'd.) Detailed reactions of cultures of Streptococcus pneumoniae from pneumonic goats.

| Culture Numbers | T4/75 | T10/75 | T19/75 | T26/75 | T35/76 | T38/76 | T41/76 | R58/76 | R66/76 |
|---|---|---|---|---|---|---|---|---|---|
| Shape | S | S | S | S | S | S | S | S | S |
| Acid fast | - | - | - | - | - | - | - | - | - |
| Spores | - | - | - | - | - | - | - | - | - |
| Motility | - | - | - | - | - | - | - | - | - |
| Growth in Air | + | + | + | + | + | + | + | + | + |
| Catalase | - | - | - | - | - | - | - | - | - |
| Oxidase | - | - | - | - | - | - | - | - | - |
| Glucose | + | + | + | + | + | + | + | + | + |
| OF | F | F | F | F | F | F | F | F | F |
| Haemolysis | a | a | a | a | a | a | a | a | a |
| Growth at 45°C | - | - | - | - | - | - | - | - | - |
| Growth at pH 9.6 | - | - | - | - | - | - | - | - | - |
| Growth in 6.5% Sodium Chloride | - | - | - | - | - | - | - | - | - |
| Growth on 10% bile | - | - | - | - | - | - | - | - | - |
| V.P. | - | - | - | - | - | - | - | - | - |
| Arginine hydrolysis | - | - | - | - | - | - | - | - | - |
| Aesculin hydrolysis | - | - | - | - | - | - | - | - | - |
| Litmus milk | AC | AC | AC | AC | AC | AC | AC | AC | AC |
| Gelatin liquefaction | - | - | - | - | - | - | - | - | - |
| Hippurate hydrolysis | - | - | - | - | - | - | - | - | - |
| CAMP test | - | - | - | - | - | - | - | - | - |
| Bile solubility | + | + | + | + | + | + | + | + | + |
| Optochin sensitivity | + | + | + | + | + | + | + | + | + |
| Glycerol | - | - | - | - | - | - | - | - | - |
| Lactose | + | + | + | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + | + | + | + |
| Mannitol | - | - | - | - | - | - | - | - | - |
| Raffinose | + | - | + | + | - | - | + | + | + |
| Salicin | - | - | - | - | - | - | - | - | - |
| Sorbitol | - | - | - | - | - | - | - | - | - |
| Sucrose | + | + | + | + | + | + | + | + | + |
| Trehalose | - | - | - | - | + | - | + | - | - |
| Arabinose | - | - | - | - | - | - | - | - | - |

AC = Acid clot in milk; S= Sphere; Coccus; a = Alpha haemolysis— - — —

Table IIc. (Cont'd) Detailed reactions of cultures of *Corynebacterium pyogenes* isolated from pneumonic goats.

| Culture Numbers | T39/76 | R47/76 | R49/76 | R52/76 | R65/76 | R67/76 | R70/76 | R73/76 | R76/76 |
|---|---|---|---|---|---|---|---|---|---|
| Shape | R | R | R | R | R | R | R | R | R |
| Acid fast | - | - | - | - | - | - | - | - | - |
| Spores | - | - | - | - | - | - | - | - | - |
| Motility | - | - | - | - | - | - | - | - | - |
| Growth in Air | + | + | + | + | + | + | + | + | + |
| Growth Anaerobically | + | + | + | + | + | + | + | + | + |
| Catalase | - | - | - | - | - | - | - | - | - |
| Oxidase | - | - | - | - | - | - | - | - | - |
| Glucose | + | + | + | + | + | + | + | + | + |
| OF | F | F | F | F | F | F | F | F | F |
| V.P. | - | - | - | - | - | - | - | - | - |
| Hydrogen sulphide | - | - | - | - | - | - | - | - | - |
| Haemolysis | + | + | + | + | + | + | + | + | + |
| Growth improved by blood | + | + | + | + | + | + | + | + | + |
| Nitrate reduction | + | + | + | + | + | + | + | + | + |
| Gelatin liquefaction | + | + | + | + | + | + | + | + | + |
| Urease | - | - | - | - | - | - | - | - | - |
| Lactose | + | + | + | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + | + | + | + |
| Mannitol | - | - | + | - | - | + | - | - | + |
| Salicin | - | - | - | - | - | - | - | - | - |
| Starch | + | + | + | + | + | + | + | + | + |
| Sucrose | + | - | - | + | + | - | - | + | - |
| Trehalose | - | - | - | - | - | - | - | - | - |
| Xylose | + | + | + | + | + | + | + | + | + |
| Indole | - | - | - | - | - | - | - | - | - |

Table IIc. (Cont'd) Detailed reactions of cultures of *Corynebacterium pyogenes* isolated from pneumonic goats.

| Culture numbers | L37/76 | L38/76 | L46/76 | L53/76 | L71/76 | T8/75 | T17/75 | T24/75 | T31/76 | T36/76 |
|---|---|---|---|---|---|---|---|---|---|---|
| Shape | R | R | R | R | R | R | R | R | R | R |
| Acid fast | - | - | - | - | - | - | - | - | - | - |
| Spores | - | - | - | - | - | - | - | - | - | - |
| Motility | - | - | - | - | - | - | - | - | - | - |
| Growth in Air | + | + | + | + | + | + | + | + | + | + |
| Growth Anaerobically | + | + | + | + | + | + | + | + | + | + |
| Catalase | - | - | - | - | - | - | - | - | - | - |
| Oxidase | - | - | - | - | - | - | - | - | - | - |
| Glucose | + | + | + | + | + | + | + | + | + | + |
| OF | F | F | F | F | F | F | F | F | F | F |
| V.P. | - | - | - | - | - | - | - | - | - | - |
| Hydrogen sulphide | - | - | - | - | - | - | - | - | - | - |
| Haemolysis | + | + | + | + | + | + | + | + | + | + |
| Growth improved by blood | + | + | + | + | + | + | + | + | + | + |
| Nitrate reduction | + | + | + | + | + | + | + | + | + | + |
| Gelatin liquefaction | + | + | + | + | + | + | + | + | + | + |
| Urease | - | - | - | - | - | - | - | - | - | - |
| Lactose | + | + | + | + | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + | + | + | + | + |
| Mannitol | - | + | - | - | + | - | - | + | - | + |
| Salicin | - | - | - | - | - | - | - | - | - | - |
| Starch | + | + | + | + | + | + | + | + | + | + |
| Sucrose | + | + | - | - | + | - | - | + | + | - |
| Trehalose | - | - | - | - | - | - | - | - | - | - |
| Xylose | + | + | + | + | + | + | + | + | + | + |
| Indole | - | - | - | - | - | - | - | - | - | - |

Table IIc. Detailed reactions of cultures of Corynebacterium pyogenes isolated from pneumonic goats.

| Culture Numbers | N791/75 | N009/75 | N664/76 | N28/76 | L4/75 | L6/75 | L11/75 | L16/75 | L25/75 | L32/76 |
|---|---|---|---|---|---|---|---|---|---|---|
| Shape | R | R | R | R | R | R | R | R | R | R |
| Acid fast | - | - | - | - | - | - | - | - | - | - |
| Spores | - | - | - | - | - | - | - | - | - | - |
| Motility | - | - | - | - | - | - | - | - | - | - |
| Growth in Air | + | + | + | + | + | + | + | + | + | + |
| Growth Anaerobically | + | + | + | + | + | + | + | + | + | + |
| Catalase | - | - | - | - | - | - | - | - | - | - |
| Oxidase | - | - | - | - | - | - | - | - | - | - |
| Glucose | + | + | + | + | + | + | + | + | + | + |
| OF | F | F | F | F | F | F | F | F | F | F |
| V.P. | - | - | - | - | - | - | - | - | - | - |
| Hydrogen sulphide | - | - | - | - | - | - | - | - | - | - |
| Haemolysis | + | + | + | + | + | + | + | + | + | + |
| Growth improved by blood | + | + | + | + | + | + | + | + | + | + |
| Nitrate reduction | + | + | + | + | + | + | + | + | + | + |
| Gelatin liquefaction | + | + | + | + | + | + | + | + | + | + |
| Urease | - | - | - | - | - | - | - | - | - | - |
| Lactose | + | + | + | + | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + | + | + | + | + |
| Mannitol | - | + | + | + | - | - | - | + | - | - |
| Salicin | - | - | - | - | + | - | - | - | - | + |
| Starch | + | + | + | + | + | + | + | + | + | + |
| Sucrose | + | - | + | + | - | + | - | - | + | + |
| Trehalose | - | - | - | - | - | - | - | - | - | - |
| Xylose | + | + | + | + | + | + | + | + | + | + |
| Indole | - | - | - | - | - | - | - | - | - | - |

Table IId. Detailed reactions of Streptococcus pyogenes isolated from pneumonic conditions in goats.

| Culture Numbers | N147/75 | N859/76 | N28/75 | N504/75 | N676/75 | N690/75 | N685/76 | N707/76 | N622/76 | N664/76 |
|---|---|---|---|---|---|---|---|---|---|---|
| Shape | S | S | S | S | S | S | S | S | S | S |
| Acid fast | - | - | - | - | - | - | - | - | - | - |
| Spores | - | - | - | - | - | - | - | - | - | - |
| Motility | - | - | - | - | - | - | - | - | - | - |
| Growth in Air | + | + | + | + | + | + | + | + | + | + |
| Growth anaerobically | + | + | + | + | + | + | + | + | + | + |
| Catalase | - | - | - | - | - | - | - | - | - | - |
| Oxidase | - | - | - | - | - | - | - | - | - | - |
| Glucose | + | + | + | + | + | + | + | + | + | + |
| OF | F | F | F | F | F | F | F | F | F | F |
| Haemolysis | b | b | b | b | b | b | b | b | b | b |
| Growth at 45°C. | - | - | - | - | - | - | - | - | - | - |
| Growth at pH 9.6 | - | - | - | - | - | - | - | - | - | - |
| Growth on 10% bile | - | - | - | - | - | - | - | - | - | - |
| Growth in 1/4000 tellurite | - | - | - | - | - | - | - | - | - | - |
| Growth in 6.5% Sodium chloride | - | - | - | - | - | - | - | - | - | - |
| Litmus milk | A | A | A | A | A | A | A | A | A | A |
| Gelatin liquefaction | - | - | - | - | - | - | - | - | - | - |
| Arginine hydrolysis | + | + | + | + | + | + | + | + | + | + |
| Hippurate hydrolysis | - | - | - | - | - | - | - | - | - | - |
| Aesculin hydrolysis | - | - | - | - | - | - | - | - | - | - |
| CAMP test | - | - | - | - | - | - | - | - | - | - |
| Bile solubility | - | - | - | - | - | - | - | - | - | - |
| Glycerol | - | - | - | - | - | - | - | - | - | - |
| Lactose | + | + | + | + | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + | + | + | + | + |
| Mannitol | - | - | - | - | - | - | - | - | - | - |
| Raffinose | - | - | - | - | - | - | - | - | - | - |
| Salicin | + | + | + | + | + | + | + | + | + | + |
| Sorbitol | - | - | - | - | - | - | - | - | - | - |
| Sucrose | + | + | + | + | + | + | + | + | + | + |
| Trehalose | + | + | + | + | + | + | + | + | + | + |
| Arabinose | - | - | - | - | - | - | - | - | - | - |

Table IId. (Cont'd) Detailed reactions of *Streptococcus pyogenes* isolated from pneumonic conditions in goats.

| Culture Numbers | L9/75 | L13/75 | L15/75 | L23/75 | L44/76 | L47/76 | L61/76 | L72/76 | L79/76 | T13/75 | T17/75 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Shape | S | S | S | S | S | S | S | S | S | S | S |
| Acid fast | - | - | - | - | - | - | - | - | - | - | - |
| Spores | - | - | - | - | - | - | - | - | - | - | - |
| Motility | - | - | - | - | - | - | - | - | - | - | - |
| Growth in Air | + | + | + | + | + | + | + | + | + | + | + |
| Growth anaerobically | + | + | + | + | + | + | + | + | + | + | + |
| Catalase | - | - | - | - | - | - | - | - | - | - | - |
| Oxidase | - | - | - | - | - | - | - | - | - | - | - |
| Glucose | + | + | + | + | + | + | + | + | + | + | + |
| OF | F | F | F | F | F | F | F | F | F | F | F |
| Haemolysis | b | b | b | b | b | b | b | b | b | b | b |
| Growth at 45°C | - | - | - | - | - | - | - | - | - | - | - |
| Growth at pH 9.6 | - | - | - | - | - | - | - | - | - | - | - |
| Growth on 10% bile | - | - | - | - | - | - | - | - | - | - | - |
| Growth in 1/4000 tellurite | - | - | - | - | - | - | - | - | - | - | - |
| Growth in 6.5% sodium chloride | - | - | - | - | - | - | - | - | - | - | - |
| Litmus milk | A | A | A | A | A | A | A | A | A | A | A |
| Gelatin liquefaction | - | - | - | - | - | - | - | - | - | - | - |
| Arginine hydrolysis | + | + | + | + | + | + | + | + | + | + | + |
| Hippurate hydrolysis | - | - | - | - | - | - | - | - | - | - | - |
| Aesculin hydrolysis | - | - | - | - | - | - | - | - | - | - | - |
| CAMP test | - | - | - | - | - | - | - | - | - | - | - |
| Bile solubility | - | - | - | - | - | - | - | - | - | - | - |
| Glycerol | - | - | - | - | - | - | - | - | - | - | - |
| Lactose | + | + | + | + | + | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + | + | + | + | + | + |
| Mannitol | - | - | - | - | - | - | - | - | - | - | - |
| Raffinose | - | - | - | - | - | - | - | - | - | - | - |
| Salicin | + | + | + | + | + | + | + | + | + | + | + |
| Sorbitol | - | - | - | - | - | - | - | - | - | - | - |
| Sucrose | + | + | + | + | + | + | + | + | + | + | + |
| Trehalose | + | + | + | * | * | + | + | + | + | + | + |
| Arabinose | - | - | - | - | - | - | - | - | - | - | - |

Table IId. (Cont'd) Detailed reactions of *Streptococcus pyogenes* isolated from pneumonic conditions in goats.

| Culture numbers | T21/75 | T25/75 | T37/76 | R48/76 | R54/76 | R60/76 | R72/76 | R75/76 | R76/76 | R77/76 | R80/76 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Shape | S | S | S | S | S | S | S | S | S | S | S |
| Acid fast | - | - | - | - | - | - | - | - | - | - | - |
| Spores | - | - | - | - | - | - | - | - | - | - | - |
| Growth in Air | + | + | + | + | + | + | + | + | + | + | + |
| Growth anaerobically | + | + | + | + | + | + | + | + | + | + | + |
| Catalyse | - | - | - | - | - | - | - | - | - | - | - |
| Oxidase | - | - | - | - | - | - | - | - | - | - | - |
| Glucose | + | + | + | + | + | + | + | + | + | + | + |
| OF | F | F | F | F | F | F | F | F | F | F | F |
| Haemolysis | b | b | b | b | b | b | b | b | b | b | b |
| Growth at 45°C | - | - | - | - | - | - | - | - | - | - | - |
| Growth at pH 9.6 | - | - | - | - | - | - | - | - | - | - | - |
| Growth on 10% bile | - | - | - | - | - | - | - | - | - | - | - |
| Growth in 1/4000 tellurite | - | - | - | - | - | - | - | - | - | - | - |
| Growth in 6.5% sodium chloride | - | - | - | - | - | - | - | - | - | - | - |
| Litmus milk | A | A | A | A | A | A | A | A | A | A | A |
| Gelatin liquefaction | - | - | - | - | - | - | - | - | - | - | - |
| Arginine hydrolysis | + | + | + | + | + | + | + | + | + | + | + |
| Hippurate hydrolysis | - | - | - | - | - | - | - | - | - | - | - |
| Aesculin hydrolysis | - | - | - | - | - | - | - | - | - | - | - |
| CAMP test | - | - | - | - | - | - | - | - | - | - | - |
| Bile solubility | - | - | - | - | - | - | - | - | - | - | - |
| Glycerol | - | - | - | - | - | - | - | - | - | - | - |
| Lactose | + | + | + | + | + | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + | + | + | + | + | + |
| Mannitol | - | - | - | - | - | - | - | - | - | - | - |
| Raffinose | - | - | - | - | - | - | - | - | - | - | - |
| Salicin | + | + | + | + | + | + | + | + | + | + | + |
| Sorbitol | - | - | - | - | - | - | - | - | - | - | - |
| Sucrose | + | + | + | + | + | + | + | + | + | + | + |
| Trehalose | + | + | + | + | + | + | + | + | + | + | + |
| Arabinose | - | - | - | - | - | - | - | - | - | - | - |

A = Acid; b = Beta haemolysis;

Table IIe. Detailed reactions of cultures of *Staphylococcus aureus* isolated from pneumonic conditions in goats.

| Culture Numbers | N091/75 | N199/75 | N859/75 | N96/76 | N859/76 | N684/76 | N691/76 | N697/76 | N204/76 | N780/76 |
|---|---|---|---|---|---|---|---|---|---|---|
| Shape | S | S | S | S | S | S | S | S | S | S |
| Acid fast | - | - | - | - | - | - | - | - | - | - |
| Spores | - | - | - | - | - | - | - | - | - | - |
| Motility | - | - | - | - | - | - | - | - | - | - |
| Growth in Air | + | + | + | + | + | + | + | + | + | + |
| Growth anaerobically | + | + | + | + | + | + | + | + | + | + |
| Catalase | + | + | + | + | + | + | + | + | + | + |
| Oxidase | - | - | - | - | - | - | - | - | - | - |
| Glucose | + | + | + | + | + | + | + | + | + | + |
| OF | F | F | F | F | F | F | F | F | F | F |
| V.P. | + | + | + | + | + | + | + | + | + | + |
| Nitrate reduction | + | + | + | + | + | + | + | + | + | + |
| Arginine hydrolysis | - | + | + | + | + | + | + | + | + | + |
| Phosphatase | + | + | + | + | + | + | + | + | + | + |
| Lactose | + | + | + | + | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + | + | + | + | + |
| Mannitol | + | + | + | + | + | + | + | + | + | + |
| Sucrose | + | + | + | + | + | + | + | + | + | + |
| Xylose | - | - | - | - | - | - | - | - | - | - |
| Arabinose | - | - | - | - | - | - | - | - | - | - |
| Haemolysis | + | - | + | - | - | - | + | - | + | + |
| Gelatin liquefaction | + | + | + | + | + | + | + | + | + | + |
| Urease | + | - | - | + | - | - | - | + | + | - |
| Coagulase | + | - | + | - | + | - | + | + | + | - |
| Pigment formation | + | - | + | + | - | + | - | + | - | + |

Table IIe. (Cont'd) Detailed reactions of cultures of Staphylococcous aureus isolated from pneumonic conditions in goats.

| Culture Numbers | N843/76 | N622/76 | N833/76 | N160/76 | N50/76 | N5/75 | L12/75 | L20/75 | L27/75 | L441/76 | L48/76 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Shape | S | S | S | S | S | S | S | S | S | S | S |
| Acid fast | - | - | - | - | - | - | - | - | - | - | - |
| Spores | - | - | - | - | - | - | - | - | - | - | - |
| Motility | - | - | - | - | - | - | - | - | - | - | - |
| Growth air Air | + | + | + | + | + | + | + | + | + | + | + |
| Growth anaerobically | + | + | + | + | + | + | + | + | + | + | + |
| Catalase | + | + | + | + | + | + | + | + | + | + | + |
| Oxidase | - | - | - | - | - | - | - | - | - | - | - |
| Glucose | + | + | + | + | + | + | + | + | + | + | + |
| OF | F | F | F | F | F | F | F | F | F | F | F |
| V.P. | + | + | + | + | + | + | + | + | + | + | + |
| Nitrate reduction | + | + | + | + | + | + | + | + | + | + | + |
| Arginine hydrolysis | + | + | + | + | + | + | + | + | + | + | + |
| Phosphatase | + | + | + | + | + | + | + | + | + | + | + |
| Lactose | + | + | + | + | + | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + | + | + | + | + | + |
| Mannitol | + | + | + | + | + | + | + | + | + | + | + |
| Sucrose | + | + | + | + | + | + | + | + | + | + | + |
| Xylose | - | - | - | - | - | - | - | - | - | - | - |
| Arabinose | - | - | - | - | - | - | - | - | - | - | - |
| Haemolysis | - | + | - | - | + | - | + | + | - | - | + |
| Gelatom ;oqiefaction | + | + | + | + | + | + | + | + | + | + | + |
| Urease | - | + | - | - | + | - | + | - | - | - | + |
| Coagulase | + | - | + | - | + | - | + | + | - | + | - |
| Pigment formation | + | + | - | + | + | - | + | + | + | - | + |

Table IIe. (Cont'd) Detailed reactions of cultures of *Staphylococcus aureus* isolated from pneumonic conditions in goats.

| Culture Numbers | L62/76 | L77/76 | T5/75 | T11/75 | T30/76 | T42/76 | R45/76 | R53/76 | R59/76 | R63/76 | R68/76 | R79/76 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Shape | S | S | S | S | S | S | S | S | S | S | S | S |
| Acid fast | - | - | - | - | - | - | - | - | - | - | - | - |
| Spores | - | - | - | - | - | - | - | - | - | - | - | - |
| Motility | - | - | - | - | - | - | - | - | - | - | - | - |
| Growth in Air | + | + | + | + | + | + | + | + | + | + | + | + |
| Growth anaerobically | + | + | + | + | + | + | + | + | + | + | + | + |
| Catalase | + | + | + | + | + | + | + | + | + | + | + | + |
| Oxidase | - | - | - | - | - | - | - | - | - | - | - | - |
| Glucose | + | + | + | + | + | + | + | + | + | + | + | + |
| OF | F | F | F | F | F | F | F | F | F | F | F | F |
| V.P. | + | + | + | + | + | + | + | + | + | + | + | + |
| Nitrate reduction | + | + | + | + | + | + | + | + | + | + | + | + |
| Arginine hydrolysis | + | + | + | + | + | + | + | + | + | + | + | + |
| Phosphatase | + | + | + | + | + | + | + | + | + | + | + | + |
| Lactose | + | + | + | + | + | + | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + | + | + | + | + | + | + |
| Mannitol | ± | + | + | + | + | + | + | + | + | + | + | + |
| Sucrose | + | + | + | + | + | + | + | + | + | + | + | + |
| Xylose | - | - | - | - | - | - | - | - | - | - | - | - |
| Arabinose | - | - | - | - | - | - | - | - | - | - | - | - |
| Haemolysis | - | + | - | - | + | + | - | - | + | - | - | + |
| Gelatin liquefaction | + | + | + | + | + | + | + | + | + | + | + | + |
| Urease | - | - | - | + | - | + | + | - | - | + | + | - |
| Coagulase | + | + | - | + | + | + | - | + | - | + | - | - |
| Pigment formation | - | + | + | - | + | - | + | - | - | - | + | + |

Table IIf. Detailed reactions of Klebsiella pneumoniae isolated from pneumonic goats.

| Culture Numbers | N730/76 | N140/76 | L23/75 | L40/76 | L65/76 | T7/75 | T16/75 | T30/76 | T33/76 | T38/76 | T42/76 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Shape | R | R | R | R | R | R | R | R | R | R | R |
| Motility | - | - | - | - | - | - | - | - | - | - | - |
| Growth in Air | + | + | + | + | + | + | + | + | + | + | + |
| Growth anaerobically | + | + | + | + | + | + | + | + | + | + | + |
| Catalase | + | + | + | + | + | + | + | + | + | + | + |
| Oxidase | - | - | - | - | - | - | - | - | - | - | - |
| Glucose | + | + | + | + | + | + | + | + | + | + | + |
| Carbohydrates (F/O) | F | F | F | F | F | F | F | F | F | F | F |
| Growth on KCN | - | - | - | - | - | - | - | - | - | - | - |
| Urease | + | + | + | + | + | + | + | + | + | + | + |
| Phenyl alanine | - | - | - | - | - | - | - | - | - | - | - |
| $H_2S$ from TSI | - | - | - | - | - | - | - | - | - | - | - |
| Lysine decarboxylase | + | + | + | + | + | + | + | + | + | + | + |
| Ornithine decarboxylase | - | - | - | - | - | - | - | - | - | - | - |
| Malonate | + | + | + | + | + | + | + | + | + | + | + |
| Adonitol | + | + | + | + | + | + | + | + | + | + | + |
| Arabinose | + | + | + | + | + | + | + | + | + | + | + |
| Duloatol | + | + | + | + | + | + | + | + | + | + | + |
| Glycerol | + | + | + | + | + | + | + | + | + | + | + |
| Lactose | + | + | + | + | + | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + | + | + | + | + | + |
| Mannitol | + | + | + | + | + | + | + | + | + | + | + |
| Raffinase | + | + | + | + | + | + | + | + | + | + | + |
| Salicin | + | + | + | + | + | + | + | + | + | + | + |
| Sorbitol | + | + | + | + | + | + | + | + | + | + | + |
| Sucrose | + | + | + | + | + | + | + | + | + | + | + |
| Trehalose | + | + | + | + | + | + | + | + | + | + | + |
| Xylose | + | + | + | + | + | + | + | + | + | + | + |
| ONPG | + | + | + | + | + | + | + | + | + | + | + |
| M R test | + | + | + | + | + | + | + | + | + | + | + |
| V.P. | - | - | - | - | - | - | - | - | - | - | - |

Table IIg. Detailed reactions of cultures of *Escherichia coli* isolated from pneumonic goats

| Culture Numbers | N13/76 | L56/76 | L74/76 | R53/76 | R63/76 | R73/76 |
|---|---|---|---|---|---|---|
| Shape | R | R | R | R | R | R |
| Motility | - | + | - | - | + | + |
| Growth in Air | + | + | + | + | + | + |
| Growth anaerobically | + | + | + | + | + | + |
| Catalase | + | + | + | + | + | + |
| Oxidase | - | - | - | - | - | - |
| Glucose | + | + | + | + | + | + |
| Growth on KCN Media | - | - | - | - | - | - |
| Growth on 4% selanite | + | + | + | + | + | + |
| Malonate | - | - | - | - | - | - |
| M.R. | + | + | + | + | + | + |
| V.P. | - | - | - | - | - | - |
| Indole | + | + | + | + | + | + |
| Gelatin liquefaction | - | - | - | - | - | - |
| Urease | - | - | - | - | - | - |
| Phenylalanine | - | - | - | - | - | - |
| $H_2S$ on T.S.I. | - | - | - | - | - | - |
| Ornithine decarboxylase | - | + | + | - | - | + |
| Arginine hydrolysis | + | - | - | - | + | - |
| Aesculin hydrolysis | - | - | + | + | - | + |
| Adonitol | - | - | - | - | - | - |
| Arabinose | + | + | + | + | + | + |
| Dulcitol | + | - | + | + | - | - |
| Lactose | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + |
| Mannitol | + | + | + | + | + | + |
| Rhamnose | - | + | + | + | + | - |
| Salicin | + | - | - | + | + | + |
| Sorbitol | - | - | + | + | - | - |

Table IIIa. Details of Experimental infection studies of P.multocida, Str.pneumoniae and C.pyogenes in laboratory mice.

| Number of strains tested. | Species of organisms tested. | Dose and Route of infection | No.of mice. | Period of observation. | Gross lesions | Site of isolation |
|---|---|---|---|---|---|---|
| N140/76 L76/76 T43/76 R80/76 | P.multocida | 0.1 ml sub-cutaneous | 6 | 56 hours.D | Only local oedema and congestion. | Heart blood, pericardial fluid and lung. |
| | | | 2 | 7 days.K | Fibrinopurulent pericarditis and partial consolidation of lungs. | Heart blood, lung and pericardial fluid. |
| N13/76 L67/76 T41/76 R66/76 | Str.pneumoniae. | 0.1 ml Intra-peritoneal | 8 | 72 hours.D | Acute peritonitis, localised suppuration and slight condition of lung. | Heart blood, pericardial fluid and lung. |
| N28/76 L71/76 T39/76 R76/76 | C.pyogenes | 0.1 ml Intra-peritoneal | 8 | 15 days.K | Nodule formation in the omentum and liver of four animals. No lesions noticed in the rest. | From the nodule in the omentum and liver. |

D = Died
K = Killed

Table IIIb. Details of experimental infection studies of *P.multocida*, *Str.pneumoniae* and *C.pyogenes* in laboratory rabbits.

| Number of strains tested. | Species of organisms tested. | Dose and route of infeetion | No.of rabbits. | Period of observation | Gross lesions | Site of isolation |
|---|---|---|---|---|---|---|
| N140/76<br>L76/76<br>T43/76<br>R80/76 | *P.multocida* | 0.5 ml sub-cutaneous | 8 | 56 hours. D | Local oedema, congestion, haemorrhagic trachitis and hyperaemia of kidney and intestine. | Heart blood, pericardial fluid and tracheal exudate. |
| N13/76<br>L67/76<br>T41/76<br>R66/76 | *Str.pneumoniae* | 0.5 ml Intra-peritoneal | 4 | 72 hours. D | Acute peritonitis, localised suppuration and spreading inflammatory lesions. | Heart blood, peritoneal fluid and lung. |
| | | | 4 | 15 days. K | Congestion at the site of injection with no other gross lesions | -- |
| N28/76<br>L71/76<br>T39/76<br>R76/76 | *C.pyogenes* | 0.5 ml Intra-peritoneal | 8 | 15 days. K | Localised small abseess in the omentum and liver of ofour rabbits. No lesions noticed in four animals. | From the abscess. |

Table IV. Details of experimental infection studies on *P.multocida*, *Str.pneumoniae*, and *C.pyogenes* in goats.

| Species of organisms | No.of animals | Group Number & Strain | Dose and route | Period of observation. | Lesions | Site of isolation |
|---|---|---|---|---|---|---|
| *P.multocida* | 432 | One L76/76 | 1.5 ml Intra-tracheal | 12 days. K | Slight tracheitis and a few consolidated areas in the lungs. Oedema involving the cardiac and diaphragmatic lobes. Consolidated areas showed thickening of the alveolar walls with slight proliferation of septal cells. Scanty cellular infiltration in the mucosa of bronchi and bronchioles. | Tracheal swab and lung. |
| | 433 | One L76/76 | 1.5 ml Intra-tracheal | 16 days. K | Patchy areas of consolidation diffusely scattered in left diaphragmatic lobe. Alveolar walls thickened. Massive peri-bronchiolar lymphoid hyperplasia in the form of nodules. | Lung. |
| | 434 | One L76/76 | 1.5 ml Intra-tradheal | 21 days. | A few focal consolidated areas on the cardiac lobes. Presence of numerous large macrophages in the alveolar walls and in the lumen. | Lung. |

Table IV. (Cont'd) Details of experimental infection studies on P.multocida, Str.pneumoniae, and C.pyogenes in goats.

| Species of organisms | No.of animals | Group number & strain | Dose and route | Period of observation. | Lesions | Site of isolation |
|---|---|---|---|---|---|---|
| Str.pneumoniae | 412 | Second L67/76 | 1.5 ml Intra-tracheal | 12 days. K | Oedema and congestion on the borders of the lung in the apical, cardiac and diaphragmatic lobes. Histologically slight congestion, oedema and scanty cellular reactions. | Lung |
| | 414 | Second L67/76 | 1.5 ml Intra-tracheal | 16 days.K | Focal consolidated area on the cardiac and diaphragmatic lobes of the right and left side of the lung. Consolidated areas showed infiltration with macrophages and fewlymphoid cells. Peribronchial lymphoid reaction, in addition scanty cellulr infiltration in the mucosa of some bronchioles. | Lung |
| | 417 | Second L67/76 | 1.5 ml Intra-tracheal | 21 days.K | Slightly firm focal areas on the right intermediate lobe and left apical lobe. The alveoli were completely free of any cellular reaction. The lymphoid reaction around the bronchi and bronchiole was still a constant feature. | Lung |

Table IV. (Cont'd) Details of experimental infection studies on *P.multocida*, *Str.pneumoniae*, and *C.pyogenes* in goats.

| Species of organisms | No.of animals | Group number & strain | Dose and route | Period of observation. | Lesions | Site of isolation |
|---|---|---|---|---|---|---|
| *C.pyogenes* | K.38 | Third L71/76 | 1.5 ml Intra-tracheal | 12 days. K | Moderately fleshy right intermediate lobe of lung. Pneumonic lesions noticed on anteroventral part of both the diaphragmatic lobes. The alveolar walls in many areas had undergone necrosis. Alveoli contained exudate consisting mainly of cell debris admixed with large intact mononuclear phagocytes. The bronchi and bronchiolar epithelium had undergone moderate hyperplasia. | Tracheal swab and lung. |
| | K.40 | Third L71/76 | 1.5 ml Intra-tracheal | 16 days. K | Consolidation on the posterior and anterior part of the cardiac lobe on theright side. Postroventral aspect of apical, entire cardiac and antrodorsal aspect of the diaphragmatic lobe on the left side also revealed pneumonic changes. Nacrotic areas are scattered in the apical part of the lung. Many of the intact alveoli had plaques of nacrotic mass. The epethelium of bronchi and bronchioles showed slight hyperplasia. | Lung. |
| | K.48 | Third L71/76 | 1.5 ml Intra-tracheal | 21 days. K | The lesions were almost similar as on the 16th day but slightly less in extent. Histologically there was more macrophage type reaction. | -- |

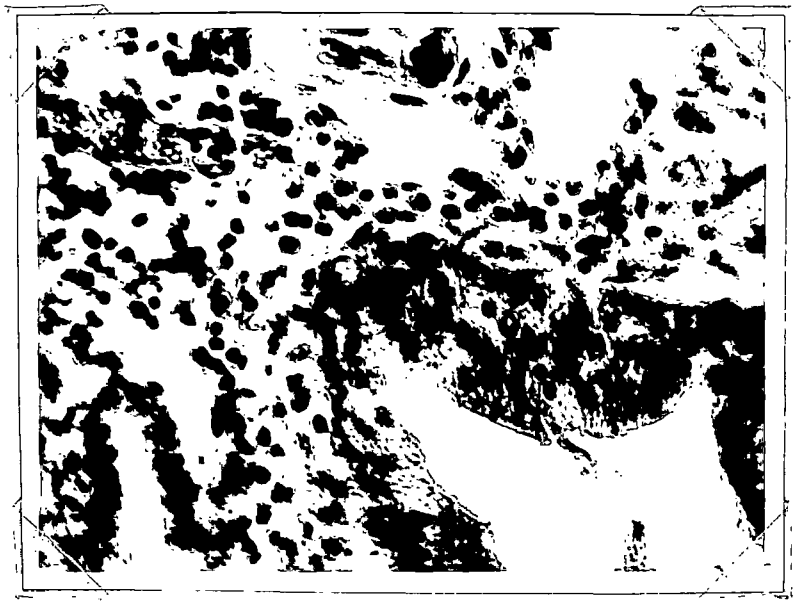
Table V. Details of the drug susceptibility of *P.multocida*, *Str.pneumoniae* and *C.pyogenes* to various chemotherapeutic agents.

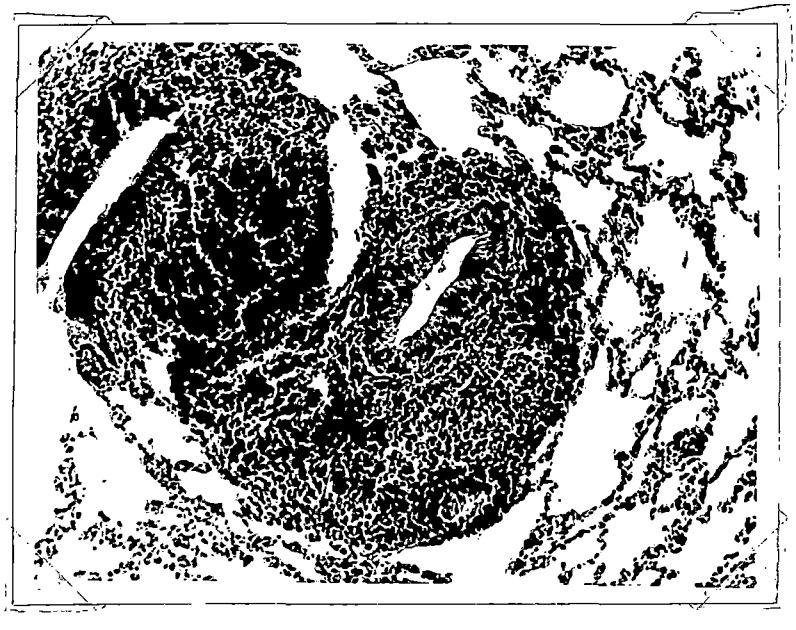
| Antibiotics | Disc contents | Organisms | No.of strains tested | No.of strains sensitive | Percentage of sensitivity |
|---|---|---|---|---|---|
| Ampicillin | 10 mcg. | *P.multocida.* | 26 | 7 | 26.92 |
| | | *Str.pneumoniae* | 19 | 16 | 84.21 |
| | | *C.pyogenes* | 29 | 29 | 100.00 |
| Bacitracin | 10 mcg. | *P.multocida* | 26 | 17 | 65.38 |
| | | *Str.pneumoniae* | 19 | 18 | 94.73 |
| | | *C.pyogenes* | 29 | 3 | 10.34 |
| Chloramphenicol | 30 mcg. | *P.multocida* | 26 | 24 | 92.30 |
| | | *Str.pneumoniae* | 19 | 19 | 100.00 |
| | | *C.pyogenes* | 29 | 29 | 100.00 |
| Erythromycin | 15 mcg. | *P.multocida* | 26 | 14 | 53.84 |
| | | *Str.pneumoniae* | 19 | 16 | 84.21 |
| | | *C.pyogenes* | 29 | 23 | 79.31 |
| Gentamycin | 10 mcg. | *P.multocida* | 26 | 15 | 57.69 |
| | | *Str.pneumoniae* | 19 | 7 | 36.84 |
| | | *C.pyogenes* | 29 | 21 | 72.41 |
| Kanamycin | 30 mcg. | *P.multocida* | 26 | 12 | 46.15 |
| | | *Str.pneumoniae* | 19 | 8 | 42.10 |
| | | *C.pyogenes* | 29 | 21 | 72.41 |

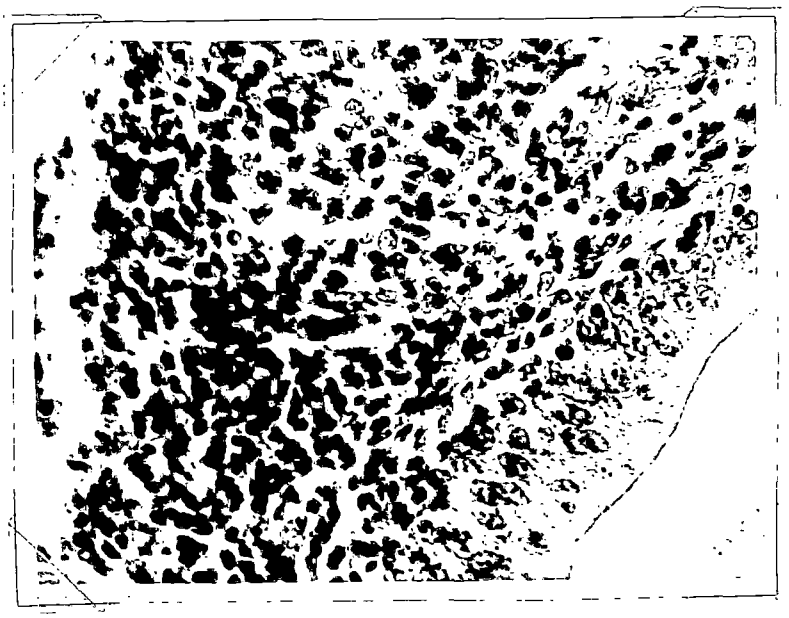
Table V. (Cont'd) Details of the drug susceptibility of *P.multocida*, *Str.pneumoniae* and *C.pyogenes* to various chemotherapeutic agents.

| Antibiotics | Disc contents | Organisms | No.of strains tested | No.of strains sensitive | Percentage of sensitivity |
|---|---|---|---|---|---|
| Nitrofurans | 300 mcg. | *P.multocida* | 26 | 26 | 100.00 |
| | | *Str.pneumoniae* | 19 | 17 | 89.47 |
| | | *C.pyogenes* | 29 | 26 | 89.65 |
| Penicillin | 10 units | *P.multocida* | 26 | 14 | 53.84 |
| | | *Str.pneumoniae* | 19 | 19 | 100.00 |
| | | *C.pyogenes* | 29 | 29 | 100.00 |
| Streptomycin | 10 mcg. | *P.multocida* | 26 | 14 | 53.84 |
| | | *Str.pneumoniae* | 19 | 3 | 15.78 |
| | | *C.pyogenes* | 29 | 0 | 0.00 |
| Tetracycline | 30 mcg. | *P.multocida* | 26 | 22 | 84.61 |
| | | *Str.pneumoniae* | 19 | 6 | 31.57 |
| | | *C.pyogenes* | 29 | 15 | 51.72 |
| Triple sulfa | 250 mcg. | *P.multocida* | 26 | 16 | 61.53 |
| | | *Str.pneumoniae* | 19 | 1 | 5.26 |
| | | *C.pyogenes* | 29 | 0 | 0.00 |

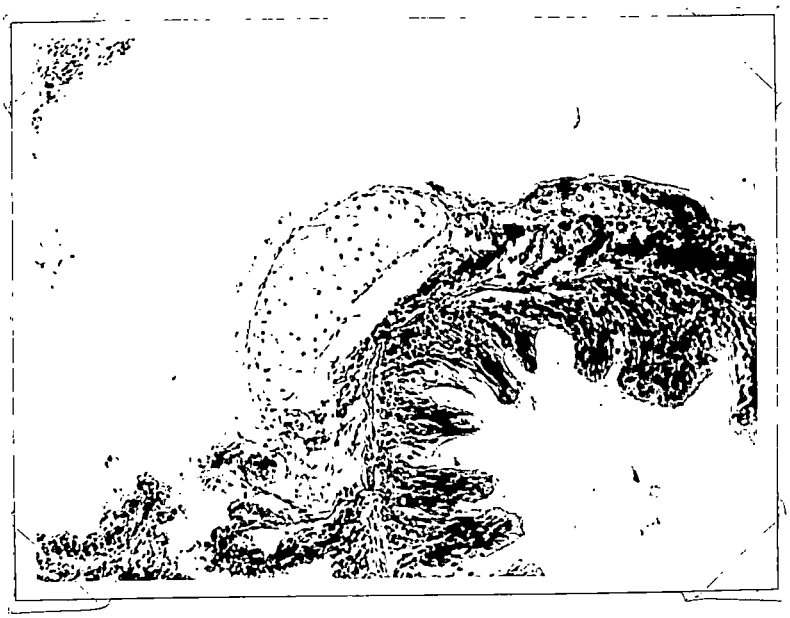
# P L A T E S

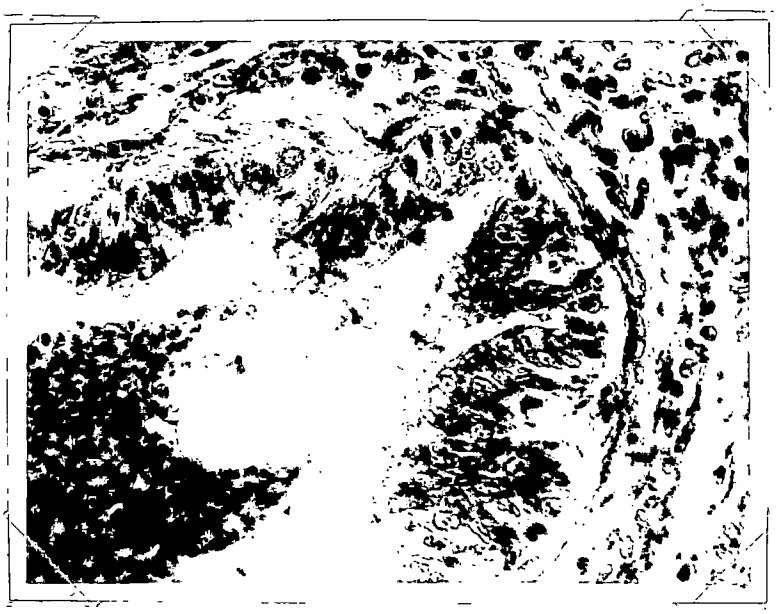


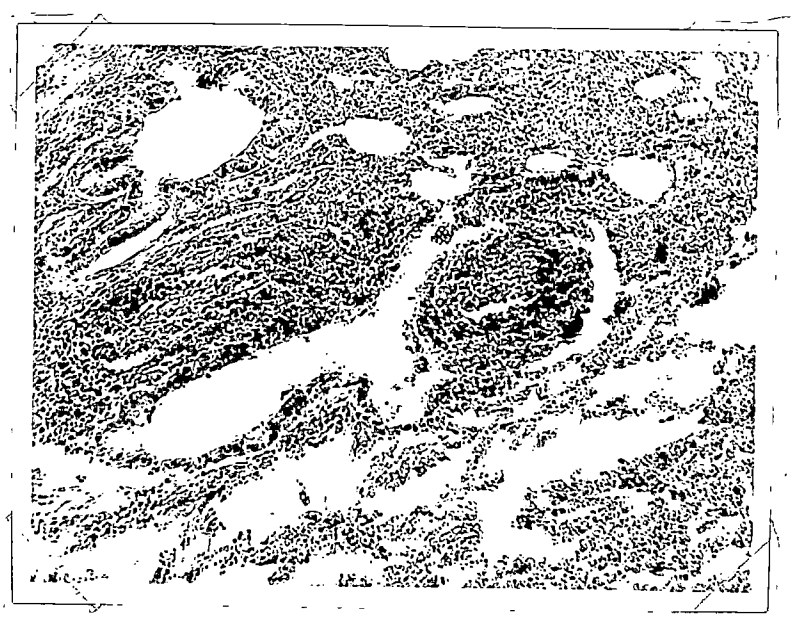


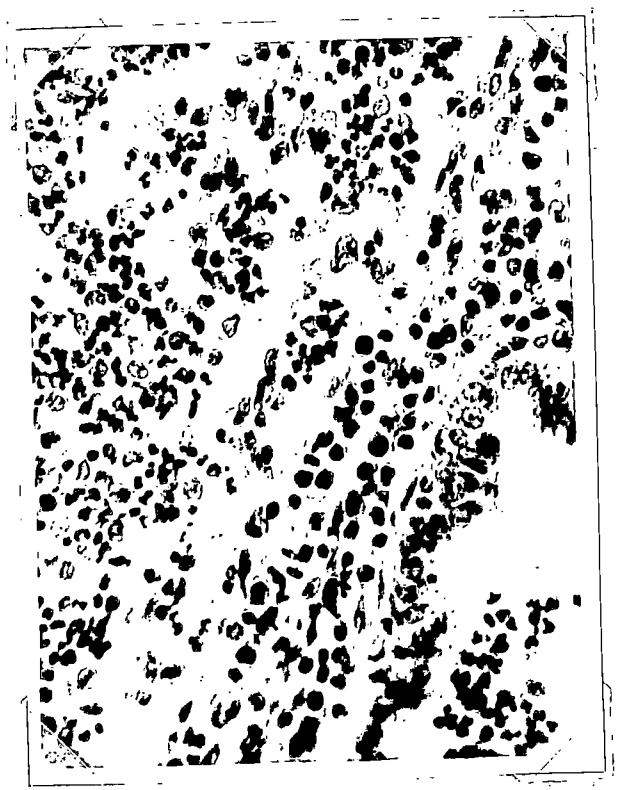












# STUDIES ON
# THE BACTERIAL SPECIES ASSOCIATED WITH
# PNEUMONIA IN GOATS

B Y

R. MADHUSOODANAN PILLAI

ABSTRACT OF A THESIS

Submitted in Partial fulfilment of the
requirement for the degree

MASTER OF VETERINARY SCIENCE

Faculty of Veterinary and Animal Sciences

Kerala Agricultural University

Department of Microbiology

College of Veterinary and Animal Sciences

Mannuthy - Trichur

1977

ABSTRACT

A total of 214 specimens which included 80 lung tissues, 55 nasal swabs, 43 tracheal swabs and 36 bronchial lymph nodes from goats with signs of pneumonia were examined during the course of this study. Out of these, 139 revealed the presence of different species of bacterial organisms. One-hundred and fifty six strains of various bacterial species were isolated and identified. The important species being Pasteurella multocida, Streptococcus pneumoniae, Streptococcus pyogenes, Corynebacterium pyogenes, Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli.

On experimental transmission study it was observed that P.multocida and Str.pneumononiae were found to be lethal to mice and rabbits whereas C.pyogenes could produce only suppurative changes in omentum and liver.

Intratracheal inoculation of the cultures of P.multocida, Str.pneumoniae and C.pyogenes in goats have revealed the reproduction of mild pneumonic changes, but did not produce death due to pneumonia. As time advanced these earlier changes showed a tendency towards healing. Compared to P.multocida and Str. pneumoniae it could be said that

C.pyogenes had produced more intense tissue reaction in the lung under identical conditions.

Invitro antibiotic sensitivity studies of P.multocida, Str.pneumoniae and C.pyogenes to 11 chemotherapeutic agents were carried out. The agents included were ampicillin, bacitracin, chloramphenicol, erythromycin, gentamycin, kanamycin, nitrofurans, penicillin, streptomycin, tetracycline and triple sulfa. In the light of the results obtained it could be stated that the drugs of choice for P.multocida are nitrofurans, chloramphenicol and tetracycline. In the case of Str.pneumoniae, penicillin, chloramphenicol and bacitracin were found to be more effective and for C.pyogenes, ampicillin, chloramphenicol and penicillin showed cent percent sensitivity.