# Aerobic Bacterial Flora of the Upper Respiratory Tract of Calves including Pasteurella

A Thesis

Submitted to Magadh University in Partial Fulfilment of the Requirements for the Degree

OF

M. Sc. (Vet.)

IN

BACTERIOLOGY

November 1965

*BY*

**S. A. Kumar,** *B. V. Sc.*

Post-Graduate Department of Bacteriology

Bihar Veterinary College, Patna

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I certify that this Thesis has been prepared under my supervision by Shri S.A.Kumar, a candidate for the Degree of M.Sc.(Vet) with Bacteriology as Major Subject, and that it incorporates the results of his independent study.

P. B. Kuppuswamy

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# C O N T E N T S



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# CHAPTER I

## Introduction

Livestock contributes very substantially towards the national income. Livestock products and animal labour for cultivation and transport are very inestimable.

In India, the profits of the farmer engaged in the Dairy-cum-Cattle industry are derived from the sale of male calves for agricultural use, breeding milch cows and sale of milk and milk products. The value of cattle sold for agricultural use or for milk production depends on the quality of the animal. The quality of the animal depends upon hereditory characters, efficient management sound nutrition and effective disease control.

Factors which considerably decrease the value of animal proteins, e.g., milk, meat, cheese etc., may result directly from bacterial, protozoal and parasitic infestations. Of these, bacterial and viral infections are major factors. A large number of acute, subacute and chronic infections due to bacteria and viruses result in degeneration or disease, affecting the quality of animal products. Much information is available on the bacterial flora

of the animal system. It would be interesting to correlate the multiplicity and the range of microbial association of the animal system and reasons for subsequent localisation of these in different areas of the body tocause the disease and consequent degeneration of animal bye-products.

The scope of present work:

It has been pointed out by the Bacteriologists and Virologists that it is necessary that the normal microbial flora of various systems should be mapped out in the way an explorer would map out the unknown land. This is necessary so that future development of the area may be possible. In the same way various grades of infection in the animal system should be worked out and understood so that exact significance of such organisms, as causative agents of diseases, could be established.

In this work, survey of the upper respiratory tract of Tharparker calves has been selected for the study, since respiratory tract next to skin and mouth offers unique opportunity to air borne bacteria to gain entry in the system.

Bacterial flora of the respiratory tract:

Respiratory system harbours bacterial flora including Pathogens, nonpathogens and intermediate types.

Although animals are constantly exposed to a set of changing environments and a wide range of infectious agents all of them do not fall sick. They expose themselves to a vast number and variety of micro-organisms but all of them do not propagate in them. A number of factors come into play in controlling the flora of a system in an animal. It is difficult to describe the basal flora of the respiratory tract common for animals living in different environments or even under similar conditions. The flora is subjected to great variations. Literature available regarding the normal flora of the respiratory tract is scanty. The importance of the work has been realised from time to time by many workers and some saliant infections have been described with special reference to organisms which are normally present in animal and man.

The organisms will not set up an infection immediately after their entrance into the system. Organisms remain in the system as nomal commensales so long as equilibrium between the virulance of the pathogens and the resistance of host exists. It is well known that the disease occurs when a susceptible animal is exposed to infection under stress and lowered resistance. Bacteria play a big role, both as

primarycausative agents of respiratory infections and also as secondary invaders in the already diseased system thereby complicating the cause of the disease. A micro-organism somewhat better able to resist the defensive mechanisms of the host but at the same time unable, except when the resistance of the host is reduced to a low level to invade the body tissues, may exist in conjunction with the host as a part of the latter's normal flora.

As long as the resistance of the host is maintained at sufficiently high level, the bacteria constituting the normal flora do not harm. It, however, the resistance is reduced, it sets up infection. The congestion of the nasal mucosa and consequent interference with ciliary activity and the movement of the mucous, which follow the temperature shock of chilling, not infrequently make possible infection by bacteria such as haemolytic streptococci or pneumococci which are already present.

Susceptability of Respiratory tract to Bacterial flora:

The bacteria and other particulate material present in inspired air are rapidly removed by nasal passages lined with mucous membrane to whose moist surface they cling. In this way air is largely freed from bacteria in the upper respiratory passages, those

that pass the larynx are caught in the bronchi and few reach ultimate ramifications of the bronchioles. The process is so efficient that expired air contains almost no bacteria except that are expelled in droplets by sneezing, coughing etc.

Many of the diseases of man and animals are transmitted as air-borne infection in which suspended infectious material is inhaled. Under natural conditions, the infectious material may occur in finely dispersed form in air originating directly from the source of infection, or it may be present in dust. It was postulated many years ago that diseases of the upper respiratory tract could be transmitted by droplets containing the micro-organisms and expelled from the mouth and nose during coughing and sneezing.

The air borne bacteria are distributed, as far as present knowledge goes, in three forms (i) attached to dust particles (ii) droplets expelled from the nose and mouth and (iii) droplet nuclei.

Dust consists varying sized particles of animal, vegetable and mineral origin. These particles carry along with them a number of micro-organisms. The heavier particles settle rapidly to the ground and those with a diameter of nearly one millimicron or less, remain more or less suspended in the atmosphere

and have every chance of reaching the respiratory passages along with the inspired air.

Animals and human beings while sneezing or coughing spray bacteria in the form of droplets as mentioned above. During a sneeze it is calculated that about 20,000 are expelled and they are sprayed to quite a distance depending upon the size of droplets (Hatch, P42). In these droplets which are larger they settle down rapidly and smaller droplets remain suspended in the air for longer time.

Droplet nuclei are formed from smaller droplets. Then smaller droplet are suspended in air for longer time. They loose water and become droplet nuclei. The droplet nuclei can be carried to longer distances by the air currents. (Wells and Luria, P41).

Abnormalities caused by Bacteria:

The bacteria that penetrate the upper respiratory passages, lodge in the bronchi and bronchioles are probably phagocytosed by the fixed alveolar epithelial cells and the wandering leucocytes that enter the bronchioles and sacs. The colonisation of bacteria in this wing in the different regions of the body are normally recognised as Pathogens, Saprophytes and Intermediates.

The following organisms are mainly concerned in one way or other with causation of pneumonia or other disease conditions. When the host-parasite relation is disturbed due to environmental factors:-

Staphylococci are the cause of 1 to 5% bacterial pneumonias. These organisms produce centrally located pulmonary lesions. Staphylococcal pneumonias occurs and indicate that the primary focus of infection elsewhere in the body (Chickening and Parks, 1919).

Certain types of E. Coli are important complicating factors in chronic respiratory diseases and other respiratory diseases (Gross, 1958). There are records to show that Escherichia species occur in respiratory tract.

Diphtheroids have been reported in cases of pneumonia practially in all species of domesticated animals. Diphtheroids mostly produce subacute to chronic form of disease, though they have also been associated with acute fatal types of pleuropneumonia in cattle and swine (Merchant and Packer, 1956). Corynebacterium has been isolated from a number of cases of enzootic form of pleuropneumonia in goats in India (Cooper, 1926, Edwards, 1926-29).

Pseudomonas aeruginosa is generally regarded as harmless saprophytes. But it has since been found that this bacterium is casually associated with a great variety of suppurative and other affections in man. Cases of endocarditis and pneumonia have also been met with as due to Pseudomonas aeruginosa.

Pneumonia due to Friedlanders bacillus makes up 0,5 to 4 percent of all pneumonias. The organims although capable of producing pneumonic complications are better recognised as secondary invaders. These micro-organisms have a greater tendency to produce necrotic lesions than Pneumococcus and the infection contrasts with pneumococcal pneumonia.

Certain types of the Streptococci are responsible for specific diseases of domestic animals. Str. equi is the cause of strangles in horses, a suppurative infection of the upper respiratory tract. Other strains of group 'C' infect cattle, horses causing respiratory catarrh and suppurative lesions in various parts of the body. The most common cause of Streptococcal mastitis in the cow is Str. agalactiae. Str. pneumoniae is normal inhabitant of lungs and in lessernumber of cases in the upper respiratory tract, of practically all species of domestic animals and man. It has been ascribed to as primary causative agent of lobar pneumonia in

man and secondary infection in domestic animals (Merchant and Packer, 1936).

Bordetella bronchiseptica originally isolated from dogs ill with distemper. It is however generally not considered as the causal organisms of the disease. It is, however, frequently found as the cause of broncho-pneumonia in guinea-pigs and other rodens. (Burrows, 1959). It is accounted as a factor in porcine pneumonia (Philips, 1943). It is also considered as secondary infection in Pneumonias and Pharyngitis of domestic animals (Laidlaw and Dunkin, 1926).

Haemophilus influenzae though not the primary cause of influenza in human beings and swine, plays a definite role in the disease through the primary cause of which is a virus (Smith, Andrews and Laidlaw, 1933).

The prevelance of Salmonella species in the respiratory tract were noted by some workers. Salmonella suipestifer pneumoniae are reported in three (human) cases. (Levine, 1944). There are records to show Salmonella typhisuis caused pneumonia in pigs (Meszaros, 1962).

Proteus bacilli are frequently found in, and appear to be responsible for a number of inflamatory and suppurative processes in man. Proteus Species were isolated from variety of conditions like croupous

pneumonia. Their carrier rate in the respiratory tract as far as the present knowledge goes is very limited (Topley, 1961).

Occurance of Bacillus actinoides in the respiratory tract and causing pneumonia were isolated. Infection by Bacillus actinoides responsible for Pneumonia was recorded. (Blake More, 1945). Bacillus actinoides may also be responsible for bronchitis in calves (Gunning, 1946).

Organisms belonging to the genus Dialister have been isolated from the respiratory tract of man and rabbits and are associated with influenza in human beings. Mention may be made of D. pneumosintes and D. granuliformus (Olitsky and Gates, 1921 - Bergey, 1957). These organism have not so far been isolated from the respiratory tract domestic animals.

The Mycobacterium tuberculosis bacilli are essentially pathogenic. The human, bovine and avian strains give rise to mammalian tuberculosis. The saprophytic acid - fast bacilli are found in such diverse surroundings as butter, milk, smegma, grass manure and faecess etc. They have been isolated in cultures made from a gangrenous lung, human faeces, tonsils, nasal secretions etc., (Topley & Wilson, 1960).

The organisms responsible for Pleuropneumonia were recognised and cultivated by Nocard and Roux as early as in 1898. The disease was reported from the North West Frontier Province, Bombay and Madras. In recent times a large number of saprophytic and pathogenic pleuropneumonia like organisms were isolated from animals and poultry. A large number of pathogenic pleuropneumonia like organisms have been isolated from birds suffering from respiratory symptoms.

Nocardia Species of organisms (belonging to the family Actinomycetales ) have been described to be associated with a large variety of affections involving pulmonary system. Mention may be made of N. Farcinica and N. asteroides in animal resembling pulmonary tuberculosis (Bergy, 1957).

Pasteurella Species have a long association in the history of pneumonia in animals. As early as 1878, Bollinger and Kitt observed epidemic of pasteurellosis in various species. The term pasteurellosis is generally used as referring to the conditions which for many years was known as Haemorrhagic Septicaemia. Bovine Pasteurellosis can be devided into 3 forms (i) Septicaemic form (ii) the oedematous form characterised by oedema of head (iii) and pectoral form characterised by broncho-

pneumonia and pleurisy. (Williams, 1932). The carrier rate of pasteurella organisms in the throat is in between 3 to 4 percent as recorded by so many observers (Singh, 1948). Mention may be made of P. multocida and P. haemolytica as causative agents of pneumonia in various species of animals.

# CHAPTER II

## Materials and Methods

The study comprised the examination of the upper respiratory tracts of one hundred and thirty five calves of Tharparkar cattle of Government Cattle Farm, Patna. All the calves from which nasal swabs were collected were normal in health and fell in the age group of 4 to 6 months.

The cotton wool swabs were prepared by winding cotton wool on a flexible copper wire. These swabs were fitted higher up near the mouth of the glass tubes so that the swab cannot touch the broth solution of 5 c.c. which was taken before the swab wire was fitted. These tubes with broth solution and swab above were sterilised in the autoclave under 15 lbs. pressure for 15 minutes. After sterilisation these swabs were used for collection of materials.

Before the swabs were taken the nostrils of the calves were cleaned with absolute alcohol. The numbers of the calves were noted to avoid repetition of taking swab material from the same animal. While an assistant was holding the calf with its head raised, the swab was gently passed as far back as possible into the post nasal space, removed and again passed into the other one. The material collected likewise

and immediately the swab inoculated into the broth solution which was kept ready below the tube for this purpose. The material adhering on the swab was thoroughly mixed with the media and these tubes were incubated in the incubator with the swab intact for 4 hours. The cultures were made on 10% sheep blood agar plate media from the broth after incubation.

The plates were examined after 24 hours and 48 hours incubation at 37° C. aerobically and after 3 days at room temperature. The growth on the media was examined for pigment production, colony characters and for haemolysis on blood agar plates. The noticeable colonies were picked up and sub-cultivated on blood agar slants.

The morphology, cultural biochemical and sugar fermention characters of the isolates were determined by the usual conventional methods and the isolates were classified according to Bergey's manual of Determinative Bacteriology, 1957.

## Studies on the cultures isolated.

1. Staphylococci:

The following criteria were employed for identification.

(a) Morphology and staining characters - Gram positive cocci in clusters were observed.

(b) Pigment production - Pigment production noticed on agar slants after 48 hours incubation at 37° C. and later kept at room and emparative at 24 hours to observe the pigmentation.

(c) Haemolysis - Haemolysis on sheep blood agar plates noticed by sub-culturing.

(d) Coagulase test - Coagulase test was carried using rabbit plasma diluted to nine times with normal saline.

2. Escherichia Species:

The identification of Escherichia species was done by Morphological cultural, biochemical and sugar tests.

(a) Morphology & staining characters - Gram negative short bacilli with parallel sides and rounded ends were seen.

(b) Cultural characters - These were studied on nutrient broth and plain agar plates. The growth on the MacConky media observed.

(c) Indol production test.

(d) Methyl red test.

(e) Test for Hydrogen Sulphide production.

(f) Nitrate reduction.

(g) Voges-Proskour reaction.

(h) Citrate utilisation test.

(i) Gelatin liquifaction.

(j) Hydrolysis of urea.

All these tests were carried as per the standard techniques.

(k) Fermentation of sugars - The sugars employed for identification of species are lactose, sucrose, glucose, maltose, mannitol and inositol.

One percent peptone water containing one percent of the sugar and Andrerd's indicator with a lyer of sterilised paraffin over it, was inoculated and incubated for 7 days at 37° C. The tubes were examined daily for acid and gas production.

(3) Klebsiella Species:

The identification and differentiation of these from Escherechia Species were based on.

(a) Morphology and staining reactions.

(b) Cultural characters studied on broth, plain agar and MacConkey medias.

(c) Motility of the organisms tested. These were non motile.

(d) Methyl red test.

(e) Voges-Proskeur reaction.

(f) Citrate utilisation.

(4) Diphtheroids:

The identification of Diphtheroids was carried by under-mentioned criteria.

(a) Morphology and Staining characters - Gram positive pleomorphic rods were seen.

(b) Cultural characters were studied on blood Tellurite media and to note any pigment production plain agar slants were used.

(c) Motility.

(d) Biochemical and sugar fermention reactions were carried as mentioned earlier. The sugars employed for study were Lactose, Sucrose, Glucose, Maltose and Mannitol.

(5) Pseudomonads:

The below mentioned characters were chosen for identification.

(a) Morphology and staining characters - Gram negative slender pleomorphic rods were seen.

(b) Motility - Highly motile organism were observed.

(c) Cultural characters - The organism were grown on agar slants and in broth solution to study the pigmentation. The pigment in broth and on agar slants became progressively fluorescant with age, when stored at room temparature.

(d) Indol production test.

(e) Fermentation of sugars - Lactose sucrose, glucose, maltose and mannitol were employed.

(f) Nitrate reduction test.

(6) Alcaligenes Species:

The identification of Alcaligenes species was based on the following criteria.

(a) Morphology and staining characters.

(b) Cultural characters - On agar slants opaque entire, non chromogenic colonies were observed. The formation of turbidity was observed.

Test for motility was done. Motile and non motile types were observed.

All the biochemical reactions and sugar fermentation tests were carried as mentioned in Escherechia species.

The sugars employed in the identification of species were Lactose, Sucrose, Glucose, Maltose, Mannitol and Inositol.

(7) Streptococci:

Study of Streptococci were based on the following.

(a) Morphology and staining characters - Gram positive Cocci in short chain were observed.

(b) Cultural characters - Growth on blood agar and glucose broth was noted. Small slightly raised circular opaque colonies were observed. Fine granular deposit was noticed in the glucose broth medium.

(c) Haemolysis on sheep blood agar. The wide zone of haemolysis of β- was noted in all the strains.

(d) Fermentation of sugars - Trehalose, Sorbitol, Mannitol, Salicin and Lactose were used for the identification of the streptococcal cultures in the work.

(e) Lancefield classification - By means of precipitation reaction with the antisera available the test was carried to assess the group to which the streptococci belong. The test was carried as below. The preparation of antigenic extract and technique of the typing followed are as follows.

HcL Extract (Lancefield) - A loopful of inoculum of the organism under study from the blood agar slant was inoculated for 48 hours at 37° C. The broth solution was centrifuged at 4000 r.p.m. for 10 minutes so that sediment was formed. The supernatant was discarded

and 0.3 ml. of N/20 Hydrochloric acid was added and placed in boiling water bath for 10 minutes. Again the tube was centrifuged at the same revolutions as mentioned above and the supernatant was taken in a separate sterilised tube. The supernatant thus taken was neutralised with N/10 NaoH using phenol red one drop. This again centrifuged and the supernatant was used for the test.

Technique - The antisera available in the Bacteriology laboratory were group A, B, C, D. A very short column of grouping sera was drawn into the short pasteure pipette to a position just below the shoulder and the end of the steam sealed off in the flame. All the four groups sera were taken in different pipettes and stuck into plasticine with the butts uppermost. The antigenic extract prickled inside the butt on to the serum. The butts were left like that for 30 minutes and the precipitation if any was noted.

(8) Bordetella Species:

The following criteria were employed for the identification of Bordetella species.

Morphology and staining characters - Short coccobacillary, gram negative forms were seen under the microscope.

Cultural characters - These organisms could grow freely in nutrient broth and nutrient agar. In nutrient broth slight turbidity noticed. On nutrient agar colonies were smooth raised, entire glistening. The organism could grow well on MacConkey's medium

Motility - The organism were found motile.

Haemolysis - Any clear zone of haemolysis was not noted.

Methylene blue reduction test - The solution was not decolourised even after 48 hours incubation. The test employed is of standard technique.

Catalase test - The test was carried 20% V/V hydrogen peroxide. Gas bubbles were produced. All the cultures were catalase positive.

The other biochemical reactions and sugar fermentation reactions were tabulated in results. The sugars employed were Lactose, Glucose, Maltose, Mannitol and Inositol.

(9) Pasteurella Species:

The under mentioned tests were employed for identification of cultures.

(a) Morphology & staining characters - Gram negative coccobacillary rods were seen. Bipolar staining of the organisms were observed by Leishmanis method of staining.

(b) Cultural characters - On blood agar plates translucent growth and mucoid in nature. In nutrient broth moderate tubidity with slight powdery deposit observed. On MacConkey plate no growth observed even after 4 days incubation.

(c) The biochemical reations observed were presented in tabular fom in results.

(d) Fermentation of sugars - Four sugars were selected to group them under Robert's Types (p47). The sugars used were Mannitol, Dulcitol, Arabinose and Xylose.

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# C H A P T E R III

## Part - A

### Review of literature with special reference to Organisms isolated.

Microbiology of respiratory infections in man and in animals can be regarded as an important study from a three dimensional view. Firstly it provides a field where a comparative assessment can be made on the distribution of bacterial flora present in human and animal population. Secondly the incidence of respiratory infections can be manifested and lastly the possibility of spread of various types of organism from man to animals and vice versa can be assessed. In pursuance of these, studies on respiratory tract of man, animals and fowls are extensively carried out.

Gibbs (1931) studied saprophytic and secondary micro organisms occuring in the respiratory tract of domestic animals. A variety of organisms including spirochoete were isolated from the respiratory tract of fowls.

Shetty (1948) studied the bacterial flora of the upper respiratory tract of apparently healthy and sick dogs observed that the incidence of staphylococci, gram negative cocci were more frequent in nasal cavity

in healthy dogs. In sick animals the frequency of Staphylococci, haemolytic and non haemolytic streptococci and Bordetella bronchiseptica was significantly higher in nasal cavities than in trachea.

Smith (1961) carried observations on the aerobic bacteria of the nose and tonsils of healthy dogs. The basal flora of the nose comprised haemolysogenic coagulase variable strains of Staph. albus and that of tonsils Pasteurella septica and alpha haemolytic Streptococci of undetermined types. Bordetella bronchiseptica was not found but Alcaligenes strains bearing a superficial resemblance to it were common.

Gurukirpal Singh etal (1965) determined the bacterial flora of nose and throat of sick dogs with clinically non respiratory infections. Pathogenic Staphylococci, non pathogenic Staphylococci micrococci, haemolytic streptococci, corynebacterium and coliform appeared to be the species more frequently encountered.

Staphylococci

Staphylococci are the normalhabitants of the respiratory tract. Many bacteriologists worked on the Pneumonia caused by Staphylococci. Staphylococci

are mainly divided into two species by the Coagulase test. Those which are coagulase positive are pathogenic and coagulase negative strains non pathogenic.

Gibbs (1931) studied the occurance of Staphylococci in the respiratory tract of dogs with other bacterial flora.

Gibbs loc. cit (1931) isolated 73.8% of Staphylococci from the respiratory tracts of domestic fowls and chickens in health and disease.

Koshelev (1939) studied on the actiological factors of Pneumonia. He found along with the other organisms cocci were also responsible for Pneumonia.

Moss (1941) classified the Staphylococci isolated from the respiratory tract of dogs. The results of his findings concluded all coagulase positive strains of Staphylococci are pathogenic but that a negative coagulase test does not exclude pathogenicity.

Shetty (1948) observed that the incidence of Staphylococci was more frequent than other bacterial flora in healthy dogs.

Rountree (1956) studied the nasal carriage of Staphylococcus aureus by various domestic and laboratory animals.

Sawhney (1959) isolated fifty three cultures of Staphylococci along with other flora out of 120 swabs taken from various parts of the respiratory tract of apparently healthy goats. The carriage incidence in his study was 62%.

Escherichia Species

Very less literature is available about the occurance of coliform bacilli in the respiratory tract of animals.

Gibbs (1931) isolated Escherichia species of intestinal origin in the respiratory tract of fowls.

Koshlev (1939) isolated B.Coli along with other organisms as an aetiological factor of pneumonia in sheep.

Dubin et-al (1943) reviewed the cases of pneumonia in man associated with B. Coli infection. He described the route by which the organisms reach the lungs in man. The most likely route considered was by aspiration and the other possibility was transference from the gastro-intestinal tract.

Gross (1958) discussed the role of E. Coli in the cause of chronic respiratory disease and certain other respiratory diseases and he stated that

certain types of E. Coli are important factors in chronic respiratory diseases.

Gurukirpal Singh et.al (1965) isolated coliform bacilli with the other bacterial flora from the nose and throat of sick dogs without respiratory infections.

Diphtheroids

Diphtheroids take part in the causation of pneumonia in the large extent.

Schimid (1933) isolated Corynebacterium pyogenes as a causative agent in a serious out break of calf pneumonia.

Merchant (1935) studied the Corynebacteria with diseases of animals. He isolated corynebacterium pseudotuberculosis Ovis, C. renalis, C. pyogenes and C. equi.

Ramboli (1940) demonstrated C. pyogenes Ovis in addition to a variety of basal organisms in tonsils of healthy sheep.

Senthili (1941) isolated C. pyogenes from gangrenous lesions arising as complications to foot and mouth disease in cattle and swine.

Flatla (1942) showed C. equi as the causative agent of pneumonia and cough in foals of 1 to 4 months

old. Holtman (1945) isolated C. equi in pure culture from purulant lesions of the lungs of a six months old calf which died from chronic pneumonia. He stated the name of the organism should be changed as it produces disease in other animals besides horse. Kawahara et.al (1949) observed C. equi infection causing suppurative pneumonia in foals. The strain isolated produced when given peros or intranasally. Harakawa (1949) isolated C. equi from the abscesses of the lungs of foals. The disease was reproduced by intranasal inoculation of the organism.

Stark et.al (1957) described bronchopneumonia in foals caused by C. equi. The authors described out-break of bronchopneumonia accomplished by respiratory catarrh and purulent abscess in lungs.

Dost et.al (1958) isolated C. diphtherial from the throat swabs of dogs.

Sawhney (1959) observed the carrier rate of corynebacterium species as 7% in the respiratory tract of apparently healthy goats.

Kalinski (1962) isolated C. ovis from the pus of abscess present in lungs. Gurukirpal Singh et.al (1965) isolated corynebacterium species with

the other bacterial flora from the respiratory tract of sick dogs.

## Pseudomonads

Much literature is not available on the pneumonia cased by Pseudomonads.

Gibbs (1931) isolated two cultures of Pseudomonas aeruginosa along with other saprophytic organism in the respiratory tract of domestic fowls.

Koshelev (1939) showed Pseudomonas pyocyanea was also responsible for causation of pneumonia in sheep.

Baker (1962) noticed deaths from acute pneumonia in pigs. Pseudomonas pyocyanea had been isolated as the causative organism.

## Klebsiella Species.

Organisms belonging to the genus Klebsiella are named after Klebs, the German bacteriologist. Although Friedlander bacillus is member of the coliform group, it has been re-accepted as coli form. It has been kept apart because of its habital but is now recognised as closely allied to Bact. aerogenes (Parr, 1939).

Sale (1947) produced experimental pneumonia in white rats by intrabronchial inoculation of the bacilli suspended in mucin. The pneumonia was lobar in type and was almost uniformly fatal and simulated the acute form of the natural disease in human beings.

Flamm (1957) isolated Klebsiella pneumonia from mice showing respiratory symptoms.

White (1957) isolated an organism of the Friedlander group which responsible for four cases of mastitis in dairy cattle. The infection spread to calves by other route than ingestion of infected milk.

Sekaraiah et.al (1957) studied on Klebsiella pneumonia infection in chicks. The organism was isolated from unabsorbed yolk of chick which died 5 days after hatching.

Landord et.al (1958) isolated Klebsiella pneumoniae from a case of canine pneumonia. The organism was isolated from the lungs of a dog.

Sawhney (1959) isolated 4.7% Klebsiella species from the respiratory tract of apparently healthy goats.

Genrden (1959) isolated 10 strain of K. pneumonia from the lungs of chicks with pullorum

disease and studied the biochemical properties.

## Alcaligenes Species

Smith (1961) isolated Alcaligenes strains belonging superficial resemblance to it from the nose and tonsils of dogs.

Girkripal Singh et.al (1965) isolated Alcaligenes species from the nose of the dogs not showing any respiratory symptoms. They isolated 3 strains after examining twenty dogs.

## Streptococci

The presence of Streptococci was first recorded by Kleb (1875) in pneumonia in man.

Gibbs (1931) isolated haemolytic streptococci from the inflamatory exudate from the cases of laryngeotracheitis in fowls.

Walaman et.al (1935) isolated streptococci differing from Str. equi. from the cases of bovine infectious bronchitis.

Appolosova (1938) observed Streptococcal pneumonia in fowls as a complication of Nuttaliasis. He assumed that the out-break was due to mixed infection and Str. pyogenes not normally pathogenic induced

disease owing to the low resistance of the animals which had been weakened by the Nuttalia invasion.

Ubertini (1939) observed Pneumococcus septicaecia (Streptococcal Pneumoniae infection) in adult bovine. Organisms were isolated from the organs of the dead animals.

Palgov et.al (1940) observed Enzootic purulant broncho pneumonia in fowls. A diplococcus similar to the Pneumococcus was isolated in all cases.

Harms (1941) obtained the Pneumococcus in almost pure culture from organs of fowls suffering from Pneumonia.

Harms loc.cit (1942) isolated Pneumococcus from 50 out 150 guinea pigs which died in three months due to pneumococcal infection. The carrier rate was noted as high as 10%.

Bosworth (1944) noted the occurance of haemolytic cocco bacilli in the nose of normal sheep and cattle which were affected with nasal catarrh.

Glaster et.al (1951 a) studied on the effect of repeated Streptococcal infections on the cardio vascular system in rats. He induced pneumonia in albino

rats by multiple inoculations of group A Streptococci into the lung.

Glaster et.al (1951 b) produced Pneumonia in white rats by instillation of β-haemolytic Streptococci intranasally. Horse Fall (1951) isolated non-haemolytic Streptococci from the respiratory tract of man.

Hammer (1953) studied on Pneumococcus infection in calves. He isolated Streptococci belonging to groups D.E.L from those cases of Pneumonia. Dhanda et.al (1958) isolated Str. Pneumoniae from lesions of lungs of sheep and goats.

Sawhney (1959) isolated Streptococci from the respiratory tract of healthy goats. The carrier rate was observed as 8 percent.

Dubedout (1958) isolated Pneumococcus from the chicks suffering from Pneumonia which caused motility upto 95% motility in flocks of chicks.

Smith (1961) isolated cultures of alpha haemolytic Streptococci from the nose and tonsils of house hold dogs.

L' Ecuyer et.al (1961) conducted microbiologic Survey of Pneumonic and normal swine lungs. Past. multocida and Streptococcus species were the principal bocteria recovered from the pneumonic lungs.

Bordetella Species

Bordetella bronchiseptica was first isolated by Ferry (1912) from dog suffering from distemper. Phillps (1943) isolated Alcaligenes (Brucella) bronchiseptical as a factor in Porcine Pneumonia in pigs. Geust (1944) isolated Brucella bronchi-septica in pure cutlure from bronchial exudate of two young cachetic pigs. Dunne et.al (1961) isolated pure cultures of Haemophilus (Bordetella) bronchiseptica from lungs of four pigs and from nasal sinuses of one pig. The primary lesions were scattered areas of broncho pneumonia in the apical, cardiac and dorsal lobes.

Pasteurella Species

The Pasteurella multocida may be carried in the animal body possibly in the throat without producing any obvious ill effects and that when the host parasitic relation is upset, the disease may be precipitated as Haemorrhagic Septicaemia. Pasteurella

pneumonia is not uncommon and different bacteriologist worked and working to find out the incidence of Pasteurella pneumonia.

Williams (1931) divided bovine pasteurellosis into three forms (i) Septicaemic form i.e. Haemorrhagic Septicaemia (ii) the Oedematous form characterised by the Oedema of the head (iii) and pectoral form characterised by broncho-pneumonia and pleurisy.

Dungall (1931) studied the bacteriology of Contagious pneumonia in sheep and found an organism roughly similar to pasteurella was the cause of the disease.

Mobb (1931) recovered P. boviseptica from the pneumonic lungs of animals at Mukteswar.

Gibbs (1931) isolated a culture resembling Pasteurella avicida from the respiratory tract of domestic fowls.

Newson et al (1932) isolated bipolar organism suspected as Pasteurella species from cases of pneumonia in sheep and cattle.

Marsh (1938) described a disease with an acute pasteurella pneumonia with Corynebacterium pyogenes as the secondary invader.

Sheuk (1938) isolated Pasteurella organism from the air passages of cats suspected that cat bites were responsible for wound infection in human beings.

Bythell (1945) noticed two forms of Pasteurella infection in bovines. In the peracute form rapid death of young calves was observed by him (Haemorrhagic Septicamia). The other form was broncho pneumonia. Pasteurella organisms were isolated in both the forms.

Pope (1946) described pneumonia due to Pasteurella organism. He isolated the organism from post mortem findings.

Singh (1948) isolated 3.5% Pasteurella septica from the live animals and 7% from the dead animals. Pasteurella organism were isolated in his work from nasal cavities of young animals

Jiriana (1952) noted Pasteurella infection characterised by pneumonia in goats. Marsh (1953) discussed Pasteurella pneumonia in sheep. This form

of pneumonia was uncommon in adult sheep but caused considerable losses in lambs.

Smith (1955) frequently isolated P. Septica from tonsils of healthy dogs and less commonly from nose.

Hamdy (1958) made observations on respiratory disease agents in lambs. He recovered P. septica and P. haemolytica from the throat swabs of healthy lambs. Hamdy et.al (1959) isolated cultures of P. septica and P. haemolytica from the pneumonic lesions in the slaughtered lambs.

Carter (1958) recovered P. multocida from affected lungs of veal calves. Sergeer (1959) isolated Pasteurella from the lungs of sheep with acute broncho pneumonia.

Sawhney (1959) studied the carrier rate of the pathogenes normally encountered in the respiratory tract of apparently healthy goats. He isolated Pasteurella 8% and concluded that Pasteurella and Streptococci were more numerous in the nasal chamber.

Pande et.al (1961) isolated Pasteurella multocida type III from sheep with pneumonia. This

is the second time this serotype has been isolated from animals in India this first having been from a cat.

L. Ecuyer et.al (1961) conducted survey for the causal organism for pneumonias in swine. Past. multocida and Streptococcus species were the principal bacteria recovered from the pneumonic lungs. Omar et.al (1962) isolated a virulant strain of Past. multocida from apparently healthy buffalo.

.....

# C H A P T E R III

## Part - B

### Present findings

One thirty five apparently healthy calves were examined in the present work and the bacterial flora of the upper respiratory tract studied with particular reference to Pasteurella species. Altogether from 135 nasal swabs examined 152 cultures were obtained. These cultures were classified into different organisms according to Bergey's Manual of Determinative Bacteriology, 1957.

The incidence of different species of organisms isolated were expressed against total number of samples examined and to total number of cultures obtained (vide Table No. 1).

## TABLE NO. 1

| | Total culture obtained | Percentage incidence in calves | Percentage against total isolates |
|---|---|---|---|
| 1. Staphylococci | 56 | 41.5 | 39.5 |
| 2. Escherichia Sps. | 30 | 22.2 | 19.7 |
| 3. Corynebacterium Sps. | 16 | 11.8 | 10.5 |
| 4. Psendomonaes. | 12 | 8.5 | 7.8 |
| 5. Klebsiella Sps. | 9 | 6.6 | 5.9 |
| 6. Alcaligenes Sps. | 7 | 5.1 | 4.6 |
| 7. Streptococci | 7 | 5.1 | 4.6 |
| 8. Bordetella Sps. | 3 | 2.2 | 1.9 |
| 9. Pasteurella Sps. | 3 | 2.2 | 1.9 |
| 10. Unidentified. | 9 | 6.6 | 5.9 |
| | 152 | | |

It will be evident from the table No. 1 that although it is observed that nasal cavity harbours a variety of bacteria, the predominance of some bacteria were more and this was in the following order, viz., Staphylococci, Escherichia Sps., Corynebacterium Sps. etc.

Staphylococci - Fifty six strains of Staphylococci were isolated. The results are presented in table No. 2.

It will be seen from the tables that classification is mainly based on Haemolysis, Pigment Production and Coagulase tests. The percentage occurance of the different strains are as follows:-

| | | |
|---|---|---|
| Golden yellow strain | - | 36.7% |
| Yellow strains | - | 17.7% |
| White strains | - | 55.6% |

The haemolysis caused by Staphylococci was of β-haemolytic type. All the golden yellow strains produced β-haemolysis. Eighty percent of the yellow strains also β-haemolytic. 9.7 percent of the white strains produced β-haemolysis.

The Staphylococci isolated were all not coagulase postive. The reason for conducting this test was to know the carrier percentage of Pathogenic Staphylococci. 36.7 percent of Golden yellow strains were

coagulase positive. 39.3 percent of colourless strains were coagulase positive. All the yellow strains were invariably coagulase negative.

## TABLE NO. 2

Results showing the typing of Staphylococci isolated.

| Sl. No. | Pigment production | Haemolysis | Coagulase test |
|---|---|---|---|
| 1. | G.Y | + | + |
| 2. | - | - | - |
| 3. | Y | - | - |
| 4. | - | - | + |
| 5. | G.Y | + | + |
| 6. | G.Y | + | + |
| 7. | Y | - | - |
| 8. | - | - | + |
| 9. | - | - | + |
| 10. | - | - | - |
| 11. | - | - | + |
| 12. | - | - | + |
| 13. | Y | - | - |
| 14. | G.Y | + | + |
| 15. | G.Y | + | + |

..... (Continued)

Table No. 2 .... (continued)

| Sl. No. | Pigment production | Haemolysis | Coagulase test |
|---|---|---|---|
| 16. | - | - | + |
| 17. | - | - | + |
| 18. | Y | - | - |
| 19. | - | - | + |
| 20. | G.Y | + | + |
| 21. | Y | - | - |
| 22. | - | - | + |
| 23. | G.Y | + | + |
| 24. | - | - | + |
| 25. | Y | - | - |
| 26. | G.Y | + | + |
| 27. | Y | - | - |
| 28. | - | - | + |
| 29. | - | - | + |
| 30. | G.Y | + | + |
| 31. | G.Y | + | + |
| 32. | - | - | + |

..... (Continued)

Table No. 2 .... (continued)

| Sl. No. | Pigment production | Haemolysis | Coagulase test |
|---|---|---|---|
| 33. | - | - | + |
| 34. | Y | - | - |
| 35. | G,Y | + | + |
| 36. | G,Y | + | + |
| 37. | Y | - | - |
| 38. | - | - | + |
| 39. | - | - | - |
| 40. | - | - | + |
| 41. | G,Y | + | + |
| 42. | - | - | - |
| 43. | - | - | + |
| 44. | - | - | + |
| 45. | - | - | + |
| 46. | G,Y | + | + |
| 47. | - | - | + |
| 48. | - | - | - |
| 49. | Y | - | - |
| 50. | - | - | + |

..... (Continued)

Table No. 2 ... (continued)

| Sl. No. | Pigment production | Haemolysis | Coagulase test |
|---|---|---|---|
| 51. | - | - | + |
| 52. | - | - | + |
| 53. | G.Y | + | + |
| 54. | - | - | - |
| 55. | - | - | + |
| 56. | - | - | - |

G. Y = Golden yellow.
Y = Yellow.

Escherichia species and Klebsiella species:

In the present study 39 cultures belonging to family Enterobacteriaceae were obtained. Of these 30 cultures belonged to Escherichia species and 9 cultures of Klebsiella pneumoniae.

The characteristic and constant feature of the organisms belonging to Escherichia species isolated was that they were cocobacillary motile organisms with rounded ends. The organisms invariably took uniformly grams stain and were gram negative.

Klebsiella species on the other hand were relatively larger organisms and they were non-motile. They were also gram negative.

The colonies of organisms on agar plates belonging to Escherichia species tended to be opaque or translucent. The colonies formed by Kelbsiella species on the other hand were larger, raised and mostly mucoid in consistancy. The mucoid character was well seen on MacConkey medium. Both the species of organisms produced turbidity with slight sediment in the broth solution.

As regards biochemical reactions are concerned it is evident from Table No. 3 that Klebsiella Pneumonia always produced Hydrogen Sulphide, whereas in organism

belonging to Escherichia species, production of Hydrogen Sulphide is always absent.

On the basis of Biochemical reactions and sugar fermentation tests the Escherichia species are identified as:-

1. E. Coli.
2. E. fruendi.
3. E. intermedia.

All the 3 species produced Indol, Nitrates reduced to Nitrites and Methyl red positive. Hydrogen Sulphide was not produced by any strain. Some strains of Escherichia fruendi were positive for vorges-Proskeur reaction. The strains of Escherichia intermedia utilised citrates. All the members of Escherichia group neither liquified gelatain nor hydrolysed urea.

Klebsiella pneumonia species always produced Hydrogen Sulphide, positive for Voges-Proskeur reaction and utilised citrates uniformly. Indol was not produced by any strain.

The sugar fermentation reactions of these organisms belonging to Enterobacteriaceae family are given in Table No. 3.

The occurance of E. Coli, E. fruendi and E. intermedia are in the order of :-

E. Coli - 40 percent
E. fruendi - 40 percent
E. intermedia - 20 percent

Results showing the typing of the members of the family Enterobacteriaceae.

TABLE NO. 3

| Sl.No. | Morphology | | Growth on MacConkey | Biochemical characters | | | | | | | | Sugar fermentations | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G.ve rods | Motility | | Indol | Hydrogen Sulphide | Nitrate reduction | M.R. test | V.P. test | Citrate utilisation | Gelatin liquifaction | Urea Hydrolysis | Lactose | Sucrose | Glucose | Maltose | Mannitol | Inositol | |
| 1. | + | + | + | + | - | + | + | - | - | - | - | AG | - | AG | A | AG | - | E.coli. |
| 2. | + | + | + | + | - | + | + | - | + | - | - | AG | - | AG | AG | AG | - | E.intermedia. |
| 3. | + | + | + | + | - | + | + | - | - | - | - | AG | A | AG | AG | AG | - | E.coli. |
| 4. | + | + | + | + | - | + | + | + | - | - | - | A | - | AG | AG | AG | - | E.fruendi |
| 5. | + | + | + | + | - | + | + | + | - | - | - | AG | - | AG | - | AG | - | E.coli. |
| 6. | + | + | + | + | - | + | + | - | - | - | - | AG | A | AG | AG | AG | - | E.coli. |
| 7. | + | - | + | - | + | + | - | + | + | - | - | - | A | AG | A | A | A | K.pneumoniae. |
| 8. | + | + | + | + | - | + | + | - | - | - | - | AG | - | AG | AG | AG | - | E.fruendi. |

.... (continued)

Table No. 3 ... (Continued)

| Sl.No. | Morphology: G-ve rods | Morphology: Motility | Growth on MacConkey | Biochemical characters: Indol | Hydrogen Sulphide | Nitrate reduction | M.R.test | V.P.test | Citrate utilisation | Gelatin liquifaction | Urea Hydrolysis | Sugar fermentations: Lactose | Sucrose | Glucose | Maltose | Mannitol | Inositol | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9. | + | - | + | - | - | + | - | + | - | - | - | AG | A | AG | A | A | A | K.pneumonia |
| 10. | + | + | + | + | - | + | + | - | - | - | - | AG | A | AG | AG | - | - | E.coli. |
| 11. | + | - | + | - | + | + | - | + | - | - | - | A | A | AG | A | A | A | K.pneumoniae. |
| 12. | + | - | + | - | + | + | - | + | - | - | - | AG | A | AG | A | A | A | K.pneumoniae. |
| 13. | + | + | + | + | - | + | + | - | + | - | - | AG | A | AG | AG | - | A | E.intermedia. |
| 14. | + | - | + | - | - | + | - | + | - | - | - | AG | A | AG | A | A | A | K.pneumoniae. |
| 15. | + | + | + | - | - | + | + | + | - | - | - | AG | - | AG | A | AG | - | E.fruendi. |
| 16. | + | + | + | + | - | + | + | - | - | - | - | AG | - | AG | A | - | - | E.coli. |

.... (continued)

Table No. 3 ... (Continued)

| Sl.No. | Morphology | | Growth on MacConkey | Biochemical characters | | | | | | | | Sugar fermentations | | | | | | Remarks. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G.ve rods | Motility | | Indol | Hydrogen Sulphide | Nitrate reduction | M.R. test | V.P. test | Citrate utilisation | Gelatin liquefac. | Urea Hydrolysis | Lactose | Sucrose | Glucose | Maltose | Mannitol | Inositol | |
| 17. | + | + | + | + | - | + | + | - | - | - | - | AG | A | AG | AG | AG | - | E.coli. |
| 18. | + | + | + | + | - | + | + | - | - | - | - | AG | A | AG | AG | - | - | E.coli. |
| 19. | + | - | + | - | + | + | - | + | + | - | - | AG | A | AG | A | A | A | K.pneumoniae |
| 20. | + | + | + | + | - | + | + | - | - | - | - | AG | - | AG | AG | AG | - | E.fruendi |
| 21. | + | + | + | - | - | + | - | - | ± | - | - | AG | A | AG | AG | AG | A | E.intermedia |
| 22. | + | + | + | + | - | + | + | - | - | - | - | AG | - | AG | AG | AG | - | E.coli. |
| 23. | + | + | + | + | - | + | + | - | - | - | - | AG | - | AG | AG | AG | - | E.fruendi. |
| 24. | + | - | + | - | - | + | + | + | - | - | - | AG | - | AG | AG | A | - | E.fruendi. |

.... (Continued)

Table No. 3 ... (Continued)

| Sl.No. | Morphology | | Growth on MacConkey | Biochemical characters | | | | | | | | Sugar fermentations | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G.ve rods | Motility | | Indol | Hydrogen Sulphide | Nitrate reduction | M.R.test | V.P. test | Citrate utilisation | Gelatin Liquefaction | Urea Hydrolysis | Lactose | Sucrose | Glucose | Maltose | Mannitol | Inositol | |
| 25. | + | + | + | - | - | + | - | - | + | - | - | AG | A | AG | AG | AG | A | E.intermedia |
| 26. | + | + | + | + | - | + | + | + | - | - | - | A | - | AG | AG | AG | - | E.fruendi. |
| 27. | + | - | + | - | + | + | - | + | + | - | - | AG | A | AG | A | A | A | K.pneumoniae. |
| 28. | + | - | + | + | - | + | + | + | - | - | - | AG | - | AG | AG | AG | - | E.fruendi. |
| 29. | + | + | + | + | - | + | + | - | - | - | - | AG | - | AG | AG | - | - | E.coli. |
| 30. | + | + | + | + | - | + | + | - | - | - | - | AG | - | AG | AG | AG | - | E.fruendi. |
| 31. | + | - | + | ± | + | - | - | + | ± | - | - | AG | A | AG | A | A | A | K.pneumoniae. |
| 32. | + | + | + | + | - | + | + | + | - | - | - | AG | - | AG | AG | AG | - | E.fruendi. |

.... (Continued)

Table No. 3 ... (Continued)

| Sl. No. | Morphology: G-ve rods | Motility | Growth on MacConkey | Biochemical characters: Indol | Hydrogen Sulphide | Nitrate reduction | M.R. test | V.P. test | Citrate utilisation | Gelatin liquefaction | Urea Hydrolysis | Sugar fermentations: Lactose | Sucrose | Glucose | Maltose | Mannitol | Inositol | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 33. | + | + | + | + | - | + | + | - | - | - | - | AG | - | AG | - | AG | - | E.coli. |
| 34. | + | + | + | - | - | + | - | - | + | - | - | AG | A | AG | AG | AG | A | E.intermedia |
| 35. | + | + | + | + | - | + | + | - | - | - | - | AG | - | AG | AG | AG | - | E.fruendi. |
| 36. | + | - | + | + | + | + | - | + | - | - | - | AG | A | AG | A | A | Δ | K.pneumoniae. |
| 37. | + | + | + | - | - | + | - | - | + | - | - | AG | Δ | AG | AG | AG | A | E.intermedia. |
| 38. | + | + | + | + | - | + | + | - | - | - | - | AG | AG | Δ | AG | AG | - | E.coli. |
| 39. | + | - | + | - | - | + | + | + | - | - | - | AG | - | AG | AG | AG | - | E.fruendi. |

+ = Positive; - = Negative; ± = Weak reaction;

A = Acid; G = Gas; Δ = Weak reaction.

Corynebacterium species:

Sixteen isolates were obtained which have been classified as belonging to Corynebacterium species. These organisms were found to be Gram positive pleomorphic rods mostly slender with tapering ends. Rods with swollen ends were also found. The organisms were found in clusters or in palisade arrangement. All the isolates were non-motile and showed black pigment on blood tellurite media. On plain agar media C. equi had grown luxuriantly and produced red pigment. Pigmentation was also observed on blood agar media.

The isolates were identified on the basis of their biochemical reactions and sugar fermentation reactions as shown in Table No. 4. The incidence of different species of Corynebacterium on the basis of isolates are:

| | | |
|---|---|---|
| C. equi | - | 31.3 percent |
| C. enzymicum | - | 25.2 percent |
| C. Pseudodiphtheriticum | - | 18.9 percent |
| C. Xerosis | - | 12.6 percent |
| C. striatum | - | 6.3 percent |
| C. hoagii | - | 6.3 percent |

## TABLE NO. 4

Results showing the study on corynebacterium species isolated.

| Sl. No. | Morphology | | | | Biochemical characters | | | | | | | | Sugar fermentations | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G+ve morphic rods | Motility | Growth on tellurite | Pigment production | Indol | Hydrogen Sulphide | Nitrate reduction | M.R. test | V.P. test | Citrate utilisation | Gelatin liquifaction | Urea hydrolysis | Lactose | Sucrose | Glucose | Maltose | Mannitol | Inositol | |
| 1. | + | - | Black | - | - | - | + | - | - | - | - | + | - | A | - | - | A | - | C. Pseudodiptheriticum. |
| 2. | + | - | Black | - | + | - | + | + | - | - | - | - | - | A | A | A | - | - | C. enzymicum. |
| 3. | + | - | Black | Red | - | - | + | - | - | - | - | - | - | A | - | - | - | - | C. equi. |
| 4. | + | - | Black | - | - | + | + | - | - | - | - | - | - | A | A | A | A | - | C. hoagii. |
| 5. | + | - | Black | - | - | - | - | - | - | - | - | - | - | A | A | A | A | - | C. xerosis. |
| 6. | + | - | Black | Red | - | - | + | - | - | - | - | - | - | - | - | - | - | - | C. equi. |
| 7. | + | - | Black | - | - | - | - | - | - | - | - | - | - | A | A | A | A | - | C. xerosis. |
| 8. | + | - | Black | - | - | - | + | - | - | - | - | + | - | - | - | - | - | - | C. Pseudodiptheriticum. |

.... (continued)

Table No. 4 ... (Continued)

| Sl.No. | Morphology | | Growth on tellurite | Pigment production | Biochemical characters | | | | | | | | Sugar fermentations | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gplemorphic rod | Motility | | | Indol | Hydrogen Sulphide | Nitrate reduction | M.R. test | V.P. test | Citrate utilisation | Gelatin liquefaction | Urea hydrolysis | Lactose | Sucrose | Glucose | Maltose | Mannitol | Inositol | |
| 9. | + | - | Black Red | | - | - | + | - | - | - | - | - | - | - | - | - | - | - | C.equi. |
| 10. | + | - | Black | | - | - | - | + | - | - | - | ± | A | A | A | A | - | - | C.striatum. |
| 11. | + | - | Black | | - | + | - | ± | + | - | - | - | - | A | A | A | - | - | C.enzymicum |
| 12. | + | - | Black | | - | - | - | + | - | - | - | + | - | A | - | - | A | - | C.Pseudodiptheriticum |
| 13. | + | - | Black | | - | - | - | + | + | - | - | - | - | A | A | A | - | - | C.enzymicum |
| 14. | + | - | Black Red | | - | - | - | + | - | - | - | - | - | - | - | - | - | - | C.equi. |
| 15. | + | - | Black Red | | - | - | - | + | - | - | - | - | - | - | - | - | - | - | C.equi. |
| 16. | + | - | Black | | - | + | - | + | + | - | - | - | - | A | - | A | - | - | C.enzymicum. |

Pseudomonads:

Twelve strains of Pseudomonads were isolated. They were Gram negative pleomorphic rods and highly motile. Green pigmentation was noted in all the strains on plain agar and in nutrient broth solution. All the strains reduced Nitrates to Nitrites. Indol was not formed by seven strains. No fermentation of Sugars noted except on glucose. All the isolates were identified as Pseudomonas aeruginosa.

Alcaligenes species:

Seven strains were isolated and identified by their cultural, biochemical and sugar fermentation reactions as Alcaligenes species. Small rods and Gram negative. Motility was observed in three strains and the rest four were non motile. Three strains reduced Nitrates to Nitrites. Three strains were catalase positive. All the strains grown well on MacConkey's medium. No carbohydrate media was attacked by any strain. The reactions were tabulated on Table No. 6.

Streptococci:

Seven strains were isolated. All showed β- haemolysis on sheep-blood agar on subculturing. The wide zone of haemolysis was noted under the microscope. After identifying them as haemolytic strains they were classified by Lancefield Method by using antisera.

## TABLE NO. 5

Results showing the typing of Pseudomonads species.

| Sl. No. | Motility | G-pleomorphic rods | Green pigment in broth | Indol test | Nitrate test | Sugar fermentations | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lactose | Sucrose | Glucose | Maltose | Mannitol | |
| 1. | + | + | + | - | + | - | - | - | - | - | Pseudomonas aeriginosa. |
| 2. | + | + | + | + | + | - | - | A | - | - | ,, |
| 3. | + | + | + | - | + | - | - | A | - | - | ,, |
| 4. | + | + | + | - | + | - | - | A | - | - | ,, |
| 5. | + | + | + | - | + | - | - | A | - | - | ,, |
| 6. | + | + | + | + | + | - | - | - | - | - | ,, |
| 7. | + | + | + | - | + | - | - | A | - | - | ,, |
| 8. | + | + | + | + | + | - | - | A | - | - | ,, |

..... (Continued)

Table No. 5 .... (continued)

| Sl. No. | Motility | G. pleomorphic rods | Green pigment in broth | Indol test | Nitrate test | Lactose | Sucrose | Glucose | Maltose | Mannitol | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 9. | + | + | + | - | + | - | - | A | - | - | Pseudomonas aeriginosa. |
| 10. | + | + | + | + | + | - | - | A | - | - | ,, |
| 11. | + | + | + | - | + | - | - | A | - | - | ,, |
| 12. | + | + | + | + | + | - | - | A | - | - | ,, |

# TABLE NO. 6

Results showing the study of the members of the Family Acromobacteriaceae.

| Sl.No. | Morphology | | Growth on MacConkey | Catalase test | Biochemical characters | | | | | | | | Sugar fermentations | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G - rods | Motility | | | Indol | Hydrogen Sulphide | Nitrate reduction | M.R. test | V.P. test | Citrate utilisation | Gelatin liquefaction | Urea hydrolysis | Lactose | Sucrose | Glucose | Maltose | Mannitol | Inositol | |
| 1. | + | Motile | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | Alcaligenes species. |
| 2. | + | - | + | + | - | - | + | - | - | - | - | - | - | - | - | - | - | - | " |
| 3. | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | " |
| 4. | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | " |
| 5. | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | " |
| 6. | + | - | + | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | " |
| 7. | + | - | + | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | " |

## TABLE NO. 7

Results showing typing of Streptococci.

| Sl. No. | Haemolysis | Lancefield classification | Sugar fermentations | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|
| | | | Tre | Sor | Maint | Salici | Lactose | |
| 1. | + | C | - | A | - | A | A | Str. zooeptidimicus |
| 2. | + | B | A | - | - | A | A | Str. agalactiae |
| 3. | + | B | A | - | - | A | A | Str. agalactiae |
| 4. | + | C | - | A | - | A | A | Str. zooepidimicus |
| 5. | + | C | A | A | - | - | A | Str. dysagalactiae. |
| 6. | + | C | - | A | - | A | A | Str. zooepidimicus. |

By this classification the seven isolates were typed into two groups 'B' and 'C' groups. Sugar fermentation reactions were also observed to type them species wise. The Streptococci were identified as:

Str. zooepidimicus - 56 percent
Str. agalactiae - 28 percent
Str. dysagalactiae - 14 percent

Bordetella species:

Three strains of Bordetella bronchiseptica were identified. They were gram negative rods and actively motile. Any clear zone of haemolysis was not noted. All the three strains reduced Nitrates to Nitritis, utilised Citrate and hydrolysed urea within 6 hours of incubation. Methylene blue was not decolourised even after forty eight hours incubation. All the three strains were highly catalase positive. No sugar was attacked except in one strain in which slight production of acid was noted in Glucose. The reactions are presented in Table No. 8.

Pasteurella Species:

Three strains of Pasteurella multocida were isolated but unluckily one strain was lost during passages after testing for biochemical reactions. The isolates were coccobacillory rods and evenly gram

negative cultures showed bipolar staining when stained by Leishman's method of staining. In broth after 24 hours incubation moderate growth with slight turbidity was noted.

The three isolates reduced Nitrates to Nitrites and produced Indol and in one strain slight colour production noted. All the three strains produced Hydrogen Sulphide, Citrate was not utilised and urea not hydrolysed. No growth was observed even after 4 days incubation MacConkey plates.

For typing into considerable groups fermenting ability of the strains were tested. One strain fermented Mannitol after seven days incubation. The other strain fermented Arabinose and Xylose. On the basis of these tests the two strains were recognised as two different types. The one which attacked Mannitol into Robert's type IV and the other which attacked Arabinose and Xylose into Robert's type I. Eventhough this classification is not so reliable, it opens the way for further typing on serological grounds.

The biochemical reactions and sugar fermentation reactions are given in Table No. 9.

The reactions in relation to identified organisms are given in the Table No. 10.

## TABLE NO. 8

Results showing the typing of Bordetella species.

| Sl.No. | Morphology | | Haemolysis | Methylene blue reduction test | Catalase test | Biochemical reaction | | | | | | | | Sugar fermentation | | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G.rods | Motility | | | | Indol | Hydrogen Sulphide | Nitrate reduction | M.R.Test | V.P.test | Citrate utilisation | Gelatin liquefaction | Urea hydrolysis | Lactose | Sucrose | Glucose | Maltose | Mannitol | Inositol | | |
| 1. | + | + | - | - | ++ | - | - | + | - | - | + | - | + | - | - | - | - | - | - | | B.bronchiseptica. |
| 2. | + | + | - | - | ++ | - | - | + | - | - | + | - | + | - | - | Δ | - | - | - | | ,, |
| 3. | + | + | - | - | ++ | - | - | + | - | - | + | - | + | - | - | - | - | - | - | | ,, |

++ = Highly positive; Δ = Weak reaction.

## TABLE NO. 9

Results showing the typing of Pasteurella species.

| Sl.No. | Morphology: G.rods | Morphology: Motility | Haemolysis | Growth on MacConkey | Biochemical reactions: Indol | Hydrogen Sulphide | Nitrate reduction | M.R. test | V.P. test | Citrate utilisation | Gelatin Liquefaction | Urea hydrolysis | Sugar fermentation: Mannitol | Dulcitol | Arabinose | Xylose. | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | G.bipolas rods. | - | - | - | + | + | + | - | - | - | - | - | A | - | - | - | P.multocida Roberts Type IV. |
| 2. | + | - | - | - | + | + | + | - | - | - | - | - | - | - | $\underline{A}$ | - | Robert's Type I. |
| 3. | + | - | - | - | + | + | + | - | - | - | - | - | Culture lost. | | | | |

A = Acid; $\underline{A}$ = Weak reactions.

## TABLE NO. 10

Reactions of unidutified organisms.

| Sl.No. | Morphology | | Biochemical reactions | | | | | | | | | Sugar fermentations | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gram's stain | Motility | Indol | Hydrogen Sulphide | Nitrate reduction | M.R. test | V.P. test | Citrate utilisation | Gelatin liquifaction | Urea hydrolysis | | Lactose | Sucrose | Glucose | Maltose | Mannitol | Inositol | |
| 1. | G+rods | Motile | - | + | - | - | - | + | - | - | | - | A | - | A | - | - | |
| 2. | G-rods | Non | + | - | - | - | - | + | - | - | | - | A | - | - | - | - | |
| 3. | G-rods | Motile | - | - | - | + | - | + | + | - | | A | A | A | A | - | - | |
| 4. | G-rods | Non | - | - | + | - | + | + | - | + | | A | AG | A | A | - | A | |
| 5. | G-rods | Non | - | - | - | - | - | - | - | - | | - | - | - | - | - | - | |
| 6. | G-rods | Motile | - | - | - | - | - | - | - | - | | - | AG | - | - | AG | - | |

..... (Continued)

Table No. 10. .... (Continued)

| Sl. No. | Morphology | | Biochemical reactions | | | | | | | | | Sugar fermentations | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gram's stain | Motility | Indol | Hydrogen sulphide | Nitrate reduction | M.R. test | V.P. test | Citrate utilization | Gelatin liquefaction | Urea hydrolysis | Lactose | Sucrose | Glucose | Maltose | Mannitol | Inositol | | |
| 7. | G-rods | Non | + | - | + | - | + | - | - | - | A | - | A | A | - | - | | |
| 8. | G-rods | Non | - | - | + | + | + | - | - | - | - | A | A | A | - | - | | |
| 9. | G-rods | Non | - | - | + | + | - | - | - | - | A | - | - | A | A | - | | |
| .... | | | | | | | | | | | | | | | | | | |

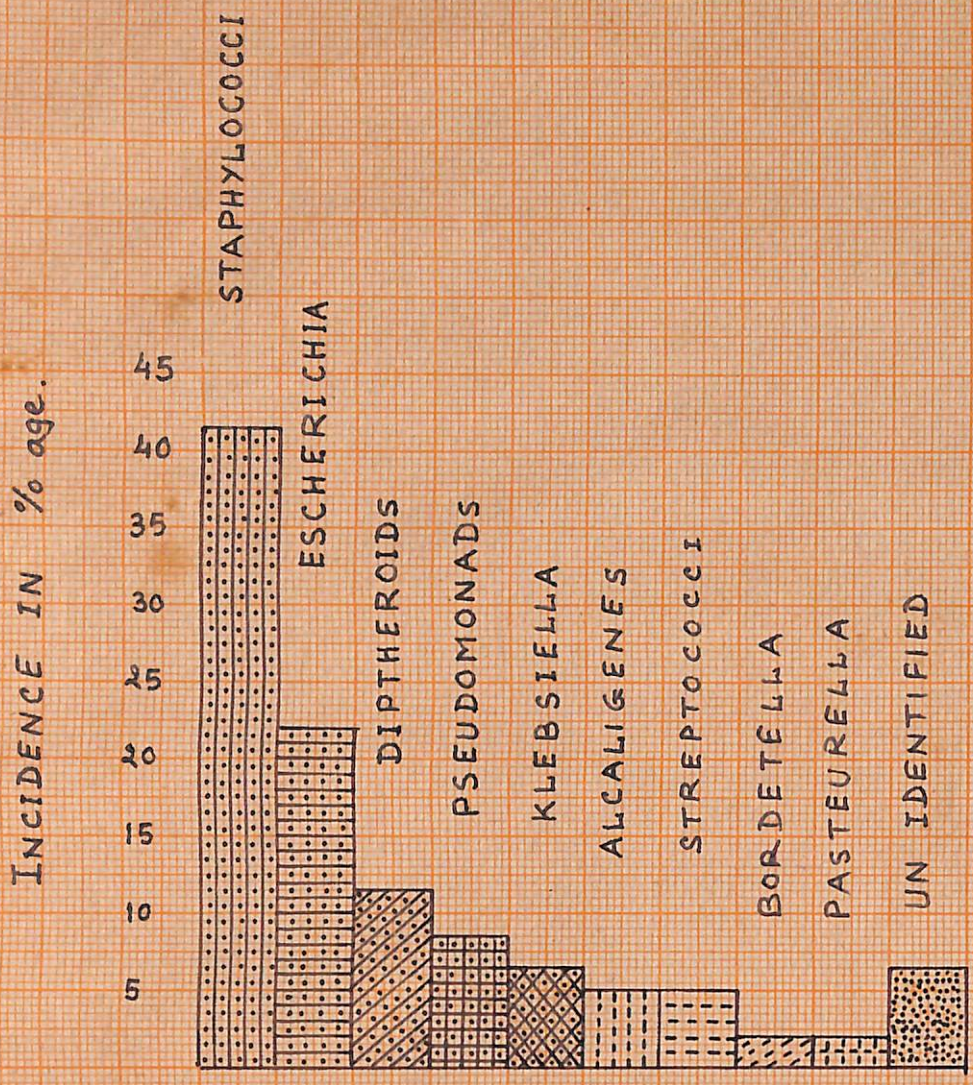


Fig. No. 1.

Histogram showing percentage incidence of different organisms in calves.

# C H A P T E R IV

## Discussion on different Organisms isolated and Conclusions.

The mucous membrane of the nares, para-nasal sinuses, pharynx, larynx and trachea are subject to injury by chemical and infectious agents brought to it in the inspired air. Injury by infectious micro-organisms including viruses is frequent and often severe. Respiratory infections among calves have been recognised as a major hazard for the successful raising of calves in India. Extensive work has been done on the carrier rate of pasteurella organisms in the respiratory tract and to assess the factors which lead to outbreaks of Haemorhagic Septicaemia.

In recent times with the awakening of interest, diseases of respiratory infections have attracted the attention of workers in the field and it is realised that systematic investigation into the cause of pneumonia and other respiratory infections and their control should be carried extensively. There are indications that some of the infections may of virus, origin such as P.L.V. group of viruses

(Matney, 1962). Aeromicrobiology had been studied extensively to get an idea on the incidence of various types of bacterial flora in man. Investigations were carried earlier on the bacterial flora of the respiratory tract in cats and dogs on account of the close association of these animals with man.

Studies on the respiratory flora in large animals had been few and fragmentary. In some instances the carrier rate has been assessed in relation to a particular disease e.g. Pasteurellosis (Singh, 1948) but a systematic data available for man and dogs, is limited.

In view of these facts, this investigation was carried on the aerobic bacterial flora of the upper respiratory tract of calves with particular reference to Pasteurella. In the present investigations following were isolated from the respiratory tract of calves:-

1. Staphylococci - 41.5 percent
2. Escherichia Sps. - 22.5 percent
3. Diphtheroids - 11.8 percent
4. Pseudomonads - 8.5 percent
5. Klebsiella Pneumoniae - 6.5 percent
6. Alcaligenes sps. - 5.1 percent

7. Streptococci - 5.1 percent

8. Bordetella bronchiseptica - 2.2 percent

9. Pasteurella multocida - 2.2 percent.

Staphylococci:

In the present study of Staphylococci were isolated from 41.5 cases. Gibbs (1931) isolated 73.8 percent of Staphylococci from the domestic animals. Sawhney (1959) recorded the percentage incidence as 62%. There is conformity with the findings of other workers who has reported similar basal flora of the nose (Shetty, 1948).

The Staphylococci isolated in the present study were grouped into Pathogenic and non Pathogenic by haemolysis and coagulase tests.

Staphylococci are responsible for suppurative lesions in cattle frequently in association with other organisms. They cause mastitis in animals and occasionally induce granulomatoues lesions in the bovine udder of chronic nature. Staphylococci are responsible for a large number of pyogenic infections in animals and man and in some cases either due to consumption of infected meat or milk, there may be severe toxaemia due to staphylococcal

enterotoxin. Septicaemia in lambs due to staphylococci is associated with ticklates. Staphyloccoci enter the body either through the respiratory tract or through the skin following breakage of the barrier by trauma. The focal necrotic form may occur due to spreading of secondary foci whatever the bacteria may lodge in. Occasionally the infection may assure fulminating bacteraemic form.

Staphylococci are normally present on the skin, in the hair water dust etc.. The staphylococci present in the air may gain enterance into the respiratory tract. A transitory drop in the resistance of these tissues may be sufficient to allow local invasion and establishment of focus of infection. Staphylococcal pneumonia usually occurs as secondary infection to influenza and bacteraemia in human beings. Staphylococci are frequently found in conjunction with the other organisms in the respiratory passages. Their exact significance of causing pneumonia in animals is difficult to assess. The factors which upset the nomal function of the respiratory tract may lead to staphylococcal pneumonia.

Escherichia Species:

Three species of Escherichia were isolated. Organisms which are placed in the genus Escherichia are widely distributed in nature and commonly found in normal intestinal tracts of man and animals. Most of these coliform bacilli appear to be non pathogenic under ordinary conditions. In the present study three species isolated are;

E. fruendi

E. intermedia

E. Coli.

Escherichia freundi is an organism most commonly found in soil and water and to some extent in the intestinal tract of man and animals. These are found in the respiratory tract also as they are widely distributed. It leads a saprophytic life in nature and is non pathogenic.

Escherichia intermedia, likewise is found in soil, water and infrequently in the intestinal tract of man and animals but not associated so far with any disease.

Certain types of B. Coli are responsible for acute enteritis in calves known as White Scours in newly born calves. The disease will be precipitated as a result of the shifting of equilibrium in favour of Bact. Coli normally present. Deprivation of colostrum, over distension of the digestive tract or some other intestinal disturbance may lead to a quantitative increase as well as virulance of the bacteria may precipitate the disease.

Pneumonia due to E. Coli is not uncommon in animals and man. E. coli may cause chronic respiratory diseases in Poultry (Gross, 1958). The isolates recovered in the present study need further study for their pathogenicity. E. coli may occur as commensal in the respiratory tract and may be carried to intestines via haematogenous route or vice versa and may cause serious troubles like "white scours" under conditions mentioned above. Further investigation is needed to find out whether the pathogenic strains leading saprophytic life are identical with the pathogenic strains of the intestines to ascertain the role of E. coli from the respiratory tract intestines. Dubin et al (1943) discussed the role of E. coli in the causation of Pneumonia and

considered the aspiration as most likely route. The other posibility is transference from the gastro intestinal tract by the blood stream. The factors which may decrease the resistance of the animal may give way for these organisms to set up Pneumonia.

Diphtheroids:

The Diptheroids organisms form a large group of bacteria which are rather common on mucous membranes of skin and animals. The isolation of diphtheroids organisms may lead to false diagnosis in some. The ubiquity of such bacteria emphasizes the need for careful study of the numerous strains of an organism before establishing aetioligic significance.

In the present study all the diphtheroids were non pathogenic organisms except for C. equi as will be mentioned later. No disease is recognised to be caused by the other diphtheroids. Their natural habitate is water, soil and mucous membranes of the animals. They live like commensols without producing any effect on the host.

C. equi was first isolated from foals as a causative organism of pneumonia. But it has been recovered from so many other animals including pig as a causative organism of pneumonia. The transmission of C. equi is not known correctly. It gains entrance presumbly through the respiratory tract although haemotogenous origin of the pulmonary infection possible. In the present study since C. equi was isolated from the normal healthy calves also it is quite likely that these organisms have an important bearing in the precipitation of pneumonia in animals when the resistance of the animal is lowered.

Pseudomonads:

Pseudomonas aeruginosa occurs from various animal and human lesions. Found in polluted water and sewage, Pseudomonas aeuriginosa is generally considered as harmless saprophyte or at the most as a micro organism of slight pathogenic power. It has since been learnt that this bacterium is associated with a great variety of suppurative and other affections in man and animals. Pseudomonas pneumonia was recorded in swine (Baker, 1962).

Pseudomonas aeruginosa itself gives rise occasionally to suppurative processes and less often to generalised infection. Among the commonest manifestations are middle ear suppuration in children, destructive lesions of the skin and nectrotic and ulcerative lesions of the alimentary mucosa.

Pseudomonas aeruginosa is found with Staphylococci, Streptococci and other micro-organisms. Its role in inciting pathological processes is problamatical. Numerous instances are in record in which little or no question exists as to its etiological role. Cases of endocarditis and pneumonia have also been met with in which Pseudomonas aeruginosa seems to be the sole responsible micro-organism.

In the present study Pseudomonads were isolated from healthy calves. Their occurance in the respiratory tract may be considered as commensals only and they may cause pneumonia and other infections when the resistance of the animal is reduced.

Klebsiella Pneumonia:

Klebsiella pneumoniae, organism are believed to be normally present in the respiratory tract of

man and animals. These group of micro organisms may be found associated with various kinds of upper respiratory diseases in man. There are instances that in most instances these bacteria may be secondary invaders in the nasopharynx in persons having chronic sinusitis or chronic lung infections such as bronchiectasis.

Klebsiella has also been associated from time to time in suppurative lesions of the various parts of the body such as liver abscesses and rarely has been found to invade the blood stream to produce Septicaemia. The bacteria have been found to be etiological agents of epidemic respiratory infections in mice (Sals et al, 1947).

In the present investigation no significance can be given to their occurance in the respiratory tract. These organism may invade the tissues and may cause serious troubles as mentioned above.

Alcaligenes species:

These species of organisms found in faeces and water. But there are instances of their occurance in the respiratory tract. They cause alkali production

and ropiness in milk. These are strictly non pathogene and in no disease, these organisms have been recognised as causative agents. These might gain access as contaminants and become established in the respiratory tract.

Streptococci:

In the present investigation the three species of Streptococci were isolated. They were Str. agalactiae, Str. dysagalactiae and Str. zooepidimicus. These three species of Streptococci are widely distributed and found wherever dairy cows are kept. The incidence of Streptococcus mastitis is high. These three species are in one way or other concerned in the causation of mastitis.

Str. agalatae, Str. dysagalactiae are not pathogenic for man and animal other than bovine, nor they have been found to be pathogenic for any other organ except mammary gland of the cow. These two organisms even though their normal habitate is udder, they might have been taken into the nasal cavity through aerial contamination and living in the respiratory tract as commensols. There are no

records to show their pathogenicity in the causation of pneumonia etc. The mode of their spreading from other tissues to the udder is yet to be studied. It is generally agreed that the common manner of spreading in cows is by the hands of milker hands. No significance can be given in the present study for these organisms except to say that they might have gained entrance through the aerial contamination, water, milk etc..

Str. zooepidimicus has been isolated from numerous types of tissues and from a variety of animals. The organism commonly produces sporadic infections in animals and however some cases of bovine mastitis have been reported. The horse appears to be the most susceptible and metritis, cervicitis and sterility in mares are caused by this organism. It also causes pneumonia in horses. But pneumonia due to this organism in other animals is not yet recognised. The present study however, indicates the possibility that these organisms might under certain conditions may cause pneumonia as isolated with other organisms.

Bordetella bronchiseptica:

Bordetella bronchiseptic is encountered frequently in broncho-pneumonia and other respiratory

infections in rodents as well as swine, dogs, cats and occasionally man. This is a secondary invader in the case of canine distemper and but is capable of setting up serious respiratory infections in the dog even in the absence of the virus. There are no records to show that it can produce any disease in bovines.

Pasteurella species:

It is long known that the pasteurella organisms are common in the upper respiratory passages of healthy cattle and invade the tissues only when the resistance of the animal is lowered and set up infections like Pneumonia and Haemorrhagic Septicaemia. (Missenard , 1934) stated that although cattle and sheep harbouring Pasteurella organisms which are culturally indistinguishable, cannot set up infection. However, the present view is that pasteurella organisms can set up infection when host parasitic relation is disturbed.

(Bain, 1961) stated that Pasteurella organisms may be localised in the nose and throat and lead to acquired immunity in cattle and buffaloes.

But it appears to be contradictory with the findings of (Omar et al , 1962) who stated that it is unlikely the carrier strains are of immunological importance. This needs, therefore, further investigations to say whether the carrier strains are having any relation to immunity or not.

Pasteurella multocida have been isolated from a wide variety of mammals and birds. The present study shows that the carrier incidence is 2.3%. It is known upto 3% of normal cattle carry Pasteurella (Singh, 1948). It is also known that in Asian countries the carrier rate may be upto 5% (Bain, 1961). The proportion of carriers detected in this work shows confirmity with the findings of others. But the carrier rate detected by taking nasal swabs may not give the actual carrier rate in animals. The reason may be that the nasal passages in bovines are very long and it is very difficult to reach the remote parts.

Conclusions:

In the present study one hundred and thirty five apparently healthy calves were examined and one hundred and fifty two isolates were obtained from

them. The isolates were typed into different organisms including pathogenic and non-pathogenic ones. Out of nine typed organisms, five were found to be pathogenic on bacteriological examinations. They are (1) Staphylococci (2) Corynebacterium equi (3) Klebsiella pneumonae (4) Streptococci (5) and Pasteurella multocida as already fully described. The present studies show that out of 135 calves examined 62 were harbouring one or more of the above mentioned pathogenic organisms. So 45.9% of the apparently healthy calves were found harbouring pathogenic organisms under natural conditions. The conditions under which these organisms could set up infection had already been discussed earlier. It is therefore considered that the present findings with reference to carrier state of the above organisms in calves are very significant in the epidemiology of the related diseases.

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# CHAPTER V

## SUMMARY.

The study comprised of examination of upper respiratory tract of one hundred and thirty five Tharparkar calves, for normal bacterial flora. The calves of the age group of 4 to 6 months were selected.

The methods employed for collection of samples and their processing for identification have been described.

The number of cultures obtained from one hundred thirty five samples were one hundred fifty two and the same were identified as per the Conventional methods of Bergey's Manual of Determinative Bacteriology, 1957. The techniques employed in the isolation and identification have been mentioned.

The predominance of bacteria in the upper respiratory tract in healthy calves examined was in

the following order:-

| | | |
|---|---|---|
| 1. Staphylococci | - | 41.5 percent |
| 2. Escherichia sps. | - | 22.2 ,, |
| 3. Corynebacterium | - | 11.8 ,, |
| 4. Pseudomonads | - | 8.5 ,, |
| 5. Klebsiella sps. | - | 6.5 ,, |
| 6. Alcaligenes sps. | - | 5.1 ,, |
| 7. Streptococci | - | 5.1 ,, |
| 8. Bordetella sps. | - | 2.2 ,, |
| 9. Pasteurella sps. | - | 2.2 ,, |

Histogram showing percentage incidence of different organisms in calves was presented.

Specific reference has been made on the carrier rate of Pasteurella species in the respiratory tract and the isolates were typed into Robert's types by sugar fermentation tests.

The presence of Pathogenic bacterial flora in the respiratory tract of apparently healthy calves and their significance in the causation of Pneumonia and other conditions were discussed.

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