Aerobic Bacterial Flora of Respiratory tract in Calves and Goats

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C E R T I F I C A T E

This is to certify that the Thesis entitled " AEROBIC BACTERIAL FLORA OF RESPIRATORY TRACT IN CALVES AND GOATS " submitted for the degree of Master of Science(Vet.) in Bacteriology to the Magadh University by Sri M. A. Nangyalai embodies the result of his independent study which he carried out under my supervision and guidance .

2.12.69.

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CONTENTS

CHAPTER - I

INTRODUCTION

Bacteria and viruses invade animals' body in various ways. The chief portal of entry are the digestive, respiratory and urinogenital tract, and the skin. The establishment of infection by the invading organisms depends on, among other factors, their virulence and number as well as on the resistance of the host. The outcome of infection is further influenced by genetic and environmental factors of animals. Infective agents are transmitted from animal to man and vice versa through the bitting insects, by direct contact between susceptible animal and the sick or carriers or indirect from surroundings contaminated by blood, secretions and excretions of diseased animals.

A thorough physical examination of respiratory apparatus is often indicated as the system is easily prone to microbial infection. Both primary and secondary infection of this system are common. In general, the primary and secondary respiratory diseases of animals are associated with infections caused by bacteria, viruses and fungi through inhalation of infective droplets or droplet nuclei. It is estimated that the optimum diameter of particles reaching lung is upto 1 micron (Brown et al, 1950). When the defence apparatus of the host gets weakened or fails under various environmental or other stress factors, the organisms penetrate and produce primary or secondary pneumonia .

Primary and secondary respiratory disease particularly pneumonia in goat, sheep, cattle are due to the following agents.

1. Pasteurella and Streptococci are the common bacterial agents that causes pneumonia and other respiratory diseases in

sheep, goat and cattle .

2. Other micro-organisms such as staphylococcus aureus Corynebacterium, Haemophilus and Klebsiella have been incriminated by many workers to be associated with respiratory infections.

3. Mycoplasma produce mostly primary pneumonia (Longley, 1940; Shirlaw, 1949; Grieg, 1955).

4. Viruses are the important causative agents of respiratory diseases in general and pneumonia in particular. (Zaki Morocos et al 1953 ; Dungworth et al 1962).

5. Helminths parasites produce pneumonia specially in goat, sheep, and other animals (Rowbali, 1954) .

Bacteria and other particulate materials are present in inspired air, but are rapidly removed during passage through the tortuous nasal apparatus 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 the ultimate ramification of the bronchioles. The process is so efficient that expired air contains almost few bacteria except those that are expelled in droplets by sneezing, coughing etc.,(Burrows,1959). Thus many of the diseases of man and animals are transmitted as air-borne infection in which the suspended infectious materials are inhaled.

The bacteria that penetrate the upper respiratory passage and get lodged in the bronchi and bronchioles are probably phagocytosed by fixed alveolar epithelial cells and the wandering leucocytes that enter the bronchioles and air sacs.

The transmission of pasteurellosis occurs by ingestion of infective materials, or by inhalation of infective droplets coughed

out by infected animals which may be clinical cases or recovered carrier. In them, the infection persists in the upper respiratory tract. Past. multocida and Past. haemolytica are highly susceptible to environmental influences and it is unlikely that mediate contagion is an important factor in the spread of the disease. When the cattle are cosely confined in damps barns the disease may spread quickly and affect a large proportion of the herd within a short time but in animals at the pasture, the rate of spread of infection may be slow.

Many workers have isolated pathogenic and non-pathogenic micro-organisms both from respiratory tract and lungs of domestic animals. It is known that the occurrence of organisms in the nasal cavities is more than that in the trachea and lungs but it is less than in mouth and digestive tract because the nasal cavities are in part protected from air borne bacteria by the anatomical features of the anterior nares.

The greater part of the inhaled bacteria appear to be arrested at or near the nasal orifices. Those that pass beyond this point adhere to the film of mucus that covers the nasal mucosa and are then swept back - in this case by the current set up by the ciliate epithilium-towards the naso-and Oro-pharynx, where they join the bacteria being swept back by suction current, from the mouth (Bloomfield, 1919) .

From the foregoing account, it is clear that many potentially pathogenic micro-organisms inhabit the respiratory tract of domesticated animals without apparent clinical syndromes. The crux of the problem is where these organisms persist in the respiratory

tract in the carrier host or when the microflora become invasive under yet little understood stress factors. The study of these microflora in different regions or respiratory apparatus is important for better understanding of the epidemiology and pathogenesis of the respiratory diseases in animals as well as in man.

In the present study, an attempt was made to determine the various aerobic micro-organisms present in respiratory tract of apparently normal cattle and goats. The results were compared at different regions of the respiratory apparatus and between those obtained in health and disease involving respiratory embarrassements.

CHAPTER II

REVIEW OF LITERATURE

The role of micro-organisms as the aetiological agent of respiratory and other infections came to be known to the bacteriologists by the 19th Century, but the development of bacteriology as a subject of scientific study dates back to the middle of 19th century and is the direct outcome of the work of Louis Pasteur .

Numerous species of bacteria belonging to different taxonomical groups have been reported to be present in the respiratory tract of domesticated animals. They are reviewed briefly below :

PASTEURELLA

Mobbs(1931), at Mukteshwar isolated Pasteurella bovisentica from the pneumonic lungs of animals.

Newson and Gross,(1932) isolated Pasteurella Organisms from cases of pneumonia in sheep and cattle .

Schenck (1933) isolated Pasteurella Organisms from the air passages of cats.

Singh (1942) isolated from respiratory tract 3.5% Past. septica from the live, and 7% from the dead cattle . He also isolated Pasteurella Organisms from the nasal cavity of young calves.

Smith(1955) isolated Past. septica from tonsil of healthy dogs, but in nose the organisms were less common.

Carter(1953) recovered Past. multocida from affected lungs of calves.

Hamdy at al (1959) isolated Pasteurella septica and Past. haemolytica from pneumonic lesions of lambs .

Sergeer(1959) isolated Pasteurella Organisms from the lungs of sheep with lesions of acute broncho-pneumonia .

Gourlay and Barber(1960) indicated that very young lambs and goats were more susceptible to Past. haemolytica than adult. They felt that the organisms were the primary pathogen in kids in which they caused pneumonia.

Pande et al(1961) isolated Past. multocida type III from sheep with pneumonia. This is the second report about the isolation of this serotype from the animals in India, the first having been isolated from a cat (Kumar, 1965) .

Moerlein et al(1961) isolated a large number of Pasteurella Organisms from the lungs of cattle showing broncho-pneumonia. Affected animals showed more Pasteurella in their nasal mucosa than did the normal animals.

L' Ecuyer et al(1961) conducted survey of the aetiological agent of pneumonia in swine. Past. multocida and strentococci were the principal bacteria which were isolated from the pneumonic lungs.

Henriksen and Jyssum (1961) described what was thought to be a variant of Pasturella haemolytica under the name Pasteurella haemolytica var . urease . This was isolated from the lung of a dead goat.

Jones(1962) isolated the organisms from the human respiratory tract and called it Past. urease .

Collier (1962) reported that cattle of all age groups may be affected by Past. multocida and Past. haemolytica but animals between 6 months and 2 years of age were more susceptible than

young calves or adults. The organisms caused pneumonia.

Omar et al(1962 a) isolated a virulent strain of Pasteurella multocida from the nasal cavity of an apparently healthy buffalo. In the same year, Omar et al (1962 b) isolated Past. multocida from 12.5% of healthy sheep. They recovered Pasteurella organisms in nasopharyngeal swabs .

Post (1962) isolated 5 strains of Pasteurellae from nasal cavities of big-horned sheep. They were carriers of Past. multocida in their nasopharynx .

Ilier et al(1963) isolated 14 strains of Past. multocida from from 27 slaughtered sheep. The organisms were obtained from the respiratory tract and from pneumonic lungs.

Schipper(1963) studied Pasteurella pneumonia which was the common form of disease in cattle in Europe, U.K. and America, but not in Australia. The morbidity and mortality in young beef cattle caused by Pasteurellae were found to be approximately 17% and 7.5% respectively.

Smith(1964) studied enzootic pneumonia in sheep as a frequent precursor of pasteurellosis, but the evidence indicated that Pasteurella haemolytica was often the primary cause of the disease in that species.

Kumar(1965) isolated 2.2% Past. multocida from the nasal cavities of healthy calves.

Biberstein et al(1966 a) showed that the causative agent of the enzootic pneumonia in sheep and goat was Past. haemolytica. They isolated the organisms from the lungs. This organisms was often the primary cause of the disease in sheep.

Biberstein et al (1966 b) isolated Past. multocida (Type A) from the lungs of sheep and goats. They indicated that this organism was usually associated with enzootic pneumonia and septicaemia in these animals, especially in very young lambs and Past. haemolytica Type 'T' with septicaemia in older lambs.

Bansel and Malik (1966) reported isolation of Past. haemolytica and Past. multocida from the lesions of lungs of sheep. He examined 747 lungs of these animals.

Mugera and Kremer (1967) isolated Past. haemolytica in Kenya goats. Acute pneumonic syndromes were noticed in 30 slaughtered goats. Two days after they had been transported from Rift Valley Province to Luzuru in Central Province, 12 were affected and all of these died within 1-6 days of the conset of symptoms. Post-mortem lesions consisted of pneumonic changes in the lungs, pleurisy and fibrinous pericarditis. Past. haemolytica was isolated which caused pneumonia and death in goats inoculated i/v, intrapulmonerily or intratracheally.

Blood and Henderson (1968) reported that pasteurellosis in goats and sheep was usually associated with infection by Past. haemolytica which caused pneumonic form of the disease. In pasteurellosis of swine, Past. multocida also caused similar pulmonary involvment but pasteurellosis in cattle which was characterized by broncho-pneumonia was caused by Past. haemolytica .

Ramchandran and Sharma (1969) reported observations on the incidence of histopathology of pneumonia of sheep and goats in India. They examined lung specimens of 58 sheep and 37 goats. In sheep, the incidence of Past. haemolytica and Past. multocida was

4.3% and 8.0% but the percentages in goats were 0.0% and 5.4% respectively.

Nandita and Rao (1969) examined 198 healthy sheep in order to determine the organisms and carrier rate of Pasteurella. They isolated 11 strains (5.65%) of Past. multocida from the materials taken from their nasal cavity of 198 normal sheep.

STREPTOCOCCI

Klebs (1875) for the first time isolated streptococci from pneumonic lung of man(Kumar 1965) .

Schutz (1883) isolated streptococci from a lesion of strangles in horse.

Waldman and Kobe (1935) isolated streptococci from bovine infectius bronchitis .

Ubertini (1939) isolated Str. pneumonia from the lungs of dead cattle.

Norms (1941) isolated pure cultures of pneumococci from pneumonic lungs in fowls .

Donald and Mann(1950) reported streptococcal pneumonia from the septicaemic cases of calves which died suddenly.

Horsfall(1951) isolated non-haemolytic streptococci from the respiratory tract of man.

Hammer(1953) isolated streptococci belonging to groups D, E and L from the pneumonic calves.

Dhanda et al(1963) isolated streptococcus pneumoniae from the lungs of sheep and goats (Kumar,1965) .

Dubedont (1958), isolated pneumococci from chicks suffering from pneumonia, the mortality rate among the chicks being up to 95 %.

Sawhney(1959) isolated streptococci from the respiratory tract of healthy goats.

Romer(1960) described infection in calves with Str. pneumonia. He stressed that this was of a great public health significance. Isolation of idential strains of the organisms from the lungs of calves dying of the disease and from the throat of their human attendants suggested inter-species transmission.

Smith(1961) isolated alpha haemolytic streptococci from the nose and tonsil of dogs.

L' Ecuyer at al(1961) carried out microbial survey of pneumonic and normal lungs of swine. They isolated Streptococci and Past. multocida from the pneumonic cases. They indicated that these organisms were principal bacteria that were recovered from the lungs.

Bryans at al(1964) found Str. equi in the nasal discharge and abscesses of horses and young pure cultures of the organisms were capable of producing the disease.

Kumar(1965) isolated Streptococci (5.1%) from the nasal cavities of healthy calves.

Skovgaard(1967) made an extensive survey of the occurrence of group G & L Streptococci in pigs and poultry. In his survey, group L streptococci were found in 56% of 127 samples of bones, poultry meat and in 100% Swabs from nasal and pharyngeal cavities of 123 slaughtered fowls. Of 25 swabs from pharyngeal and laryngeal cavities of pigs, 20 yielded haemolytic streptococci of which 12 belonged to group L. Swabs from the tonsillar sinus of 17 cattle yielded 3 strains of haemolytic streptococci.

Ramchandran and Sharma (1969) isolated Str. pyogenes to the extent of 14.2% from sheep and 16.3% from goats. Str. pneumonia

were found to 11.4% in sheep and 10.8% in goats.

STAPHYLOCOCCI

Gibbs (1931) isolated staphylococci from the respiratory tract of fowls. He recovered 37.8% of staphylococci from domesticated fowls.

Gillespie et al(1939) isolated staphylococci from respiratory tracts of many normal cases, both from animal and man.

Rountree et al (1951) isolated staphylococci from the respiratory tract of various healthy domestic and laboratory animals.

Sawhney (1959) isolated 53 cultures of staphylococci along with other organisms out of 120 swabs taken from various parts of the respiratory tract of apparently healthy goat. He indicated that the carriers' incidence was 62% .

Singh (1965) isolated 60% staphylococci among the other organisms from the nose of dogs. He found the dogs to be carriers of pathogenic staphylococci .

Weston (1965) showed the incidence of the Staph. aureus in the enzootic streptococcosis of lambs. He isolated Staph. aureus from the lesions of effected flock. A large number of lambs carried staphylococci in their nasal mucosa.

Kumar (1965) isolated 41.5% of staphylococci from the nose of the healthy calves.

Bansel and Malik (1966) reported bacterial and viral agents in the lesions of lungs of sheep. They examined lungs of 747 sheep during the period of 6 months. They isolated the micro-organisms from the lungs of 102 out of 747 sheep. All these sheep were showing

pneumonic lesions. They reported 69 strains of Staph. citreus. E.coli, Klebsiella, Bacterium alkaligens, Pasteurella haemolytica. Past. multocida, Pseudomonas, Corynebacterium pyogenes and other spore-forming aerobic gram-positive bacilli. The percentage of staphylococci in the lungs of sheep was 13.3% .

Elliot (1963) surveyed the incidence of staphylococci in cows in one herd and found 14% staph. aureus in the vagina ,5.5% in the rectum and 4.8% in nose. They used selenite egg yolk media for their isolation.

Silberg (1966) studied the relative incidence of staphylococcal infection in human beings and animals. No significant difference in the incidence of nasal infection due to coagulase-positive staphylococci could be demonstrated between the faculty and staff members exposed to the Veterinary Hospital and clinics, and those who were not thus exposed. Among hospitalised animals, 20% had asymptomatic nasal infection. The overall incidence of nasal infection among out- patients animals was 29% against 34% in hospital animals.

Ramchandran et al(1969) isolated 21.4% Staphylococci aureus from sheep and 27.0% from goats . The numbers of sheep 983 and goats 1755 examined by him . All of them had suffered from pneumonia .

ESCHERICHIA COLI

Koshlev (1939) attributed Bacterium coli in association with other organisms as the aetiological agent of pneumonia in sheep.

Singh (1965) isolated Coliform bacilli in association with other bacterial flora from the nose and throat of sick dogs without respiratory infection.

Kumar (1966) isolated 22.5% of Escherichia organisms from the nares of healthy calves.

Bansel et al(1966) reported E.coli from the lung lesion of sheep in a flock of 747 sheep. E.coli were isolated from 102 of these animals.

Belchev et al(1967) studied on the aetiology of enteritis in new born calves in Bulgaria. They examined a total of 353 specimens obtained from calves that had died between 1 and 10 days of age on 20 farms. About 43% of the calves had toxic enteritis associated with the mixed infection of E.coli and Diplococcus pneumonia, Proteus and Streptococci. Pure cultures of E.coli were isolated from the nasal mucosa of 30% of calves. Cl. perfringens type B was recorded from 6.5% of calves. No fungi or viruses were isolated.

SALMONELLA

Lawson and Dow (1968) isolated Salmonella organisms from the lungs of pigs. The most frequently observed lesions in 96 pigs dying as a result of infection with Sal. choleraesuis, were purplish red discolouration of the ear, limbs and abdomen, spleenomegaly, hepatomegaly and pulmonary haemorrhage. The organisms were most consistently isolated from lungs, livers, spleen, gall bladder and kidney.

Blood and Henderson (1968) isolated Sal. abortusequi and Sal. typhimurium from lungs of feetus, but they rarely caused serious involvement of lungs .

OTHER MICRO-ORGANISMS

Schimid (1933) isolated Corynebacterium pyogenes and attributed

it to be the causative agent of calf pneumonia.

Fletla(1942) isolated C.equi from the lungs of foals 1-4 months old suffering from cough and pneumonia .

Bosworth and Lovell (1944) isolated haemolytic cocco-bacilli from the nasal cavity of goats, cattle, and sheep affected with nasal catterrh .

Holtman (1945) isolated pure culture of C.equi from purulent lesions in the lung of 6 months old calf which died from chronic pneumonia .

Harakawa (1949) isolated C. equi from the pneumonic lungs of foals.

Ludford et al(1953) isolated Klebsiella pneumonia from the lungs of a dog.

Sawhney (1959) isolated 4.7% klebsiella species from the respiratory tracts of healthy goats .

Kalinski (1962) isolated Coryne bacterium ovis from the pus of abscess present in lungs of cattle and sheep.

Baker(1962) noticed deaths from acute pneumonia in pigs. Pseudomonas pyogenes was found to be the causative organisms.

Pande et al(1962) isolated P.P.L.O. from the respiratory tract of fowls.

Molealal et al(1963) for the first time reported the presence of Aspergillus species in the respiratory tract of a cow. They described a case of pulmonary aspergillosis in a 4 years old cow. They indicated however that Aspergillus had been frequently recognised as a disease entity in young chicks and poults .

Singh and Parmaik (1965) found coliform bacilli and Coryne bacterium species among the bacterial flora of the respiratory tract of sick dogs without showing respiratory infections. They also isolated Alkeligenes species from the nasal swabs of dogs but they did not show any sign of respiratory infection.

Kardevan (1966) studied on histopathological lesions caused by Aspergillus fumigatus in experimentally infected rabbits. For this purpose, 6 rabbits of 2.5 kg.- 3 kg. body weight were infected i/v with spores of Asp. Fumigatus isolated from a turkey poult. All six rabbits developed generalised aspergillosis with granulomatous lesions involving kidney, wall of the large intestine, lungs, liver, spleen and in a few cases the brain. This indicated that A.fumigatus produced pulmonary infection in rabbits.

Bain(196) described pneumonia in Foal caused by corynebacterium. The organisms were localised in many organs particularly in lungs and produced lesions with clinical manifestations of pneumonia. He indicated that incidence was more common in foals of 1-2 months of age but it may occur in foal of up to 6 months age.

Corrado(1967) described an outbreak of a fatal respiratory infection in a flock of about 50 goats and 100 sheep in poor condition with severe helminthiasis kept in a dusty shed and fed dry forage. Diplococcus pneumonia were isolated from the lungs of 6 dead animals examined. The agent was pathogenic for mice and rabbits, but not for guinea pigs.

Skovgaard (1968) described the incidence of haemolytic bacteria in cattle with a special references to Corynebacterium Pyogenes. He studied swabs samples taken before and after slaughter

and reported the presence of C. pyogenes in the tonsilar sinus of about 108 cows, nasal cavity of 11 of 130, conjunctival sac of 4 of 107 and retropharyngeal lymph nodes of 1 of 72 animal. The organisms were present in the nasal cavity, conjunctive and vagina of 34 of 144 cows in 12 herds where summer mastitis was present, 14 of 41 clinically healthy heifers in 3 herds and 27 of 67 cows with summer mastitis were examined in this study.

Dwivedi(1958) isolated fungi from the pneumonic lungs of 43 adult buffaloes in Uttar Pradesh. Lesions were non-suppurative in 15 and suppurative in 21 cases. Mixed granulomatous pneumonia with actinobodies were present in 7 cases, in which one was of Actinomyces species and the rest had mixed infection with Aspergillus. The lesions were comparable with the pulmonary aspergillosis in calves. The lungs of 43 adult buffaloes slaughtered revealed 16 acute, 13 sub-acute and 14 chronic type of pneumonic changes.

Ramchandran and Sharma (1959) carried bacteriological and histopathological study of pneumonia in sheep and goats in India. Of 393 sheep necropised at the Indian Veterinary Research Institute Mukteshwar, 19.66% showed pneumonic lesion. Similarly, 507 of 1755 goats necropised revealed lung lesion, the incidence in them being 28.22%. At the Government Livestock Farms in the southern India, 14%, 15% and 17.2% of the total deaths among ovines in 1953, [illegible] 21% from [illegible] 27.9% from goats, following organisms were isolated from pneumonic lungs of 58 sheep and 37 goats with their percentage rates. The organisms isolated were

Streptococcus pyogenes 14.2% from sheep and 10.8% from goats, Klebsiella pneumonia 11.2% in sheep and 11.5% from goats, Corynebacterium pyogenes 27.3% from sheep and 27.1% from goats; Haemophillus 0.0% from sheep and 2% from goat and Listeria monocytogenes 1.4% only from sheep and none from goats .

CHAPTER III

MATERIAL AND METHODS

To determine the different micro-organisms in the respiratory tracts, 82 calves of 3 to 6 months age of Government Cattle Farm, Patna and 132 goats aged 4-6 months of Biological Products Section of Livestock Research Station, Patna, were taken for the study. The goats were supplied by the contractor for the preparation of Freeze Dried Rinderpest Goat Tissue (F.D.G.T.V.) vaccine.

Preparation of Swabs :-

The swabs were prepared by winding cotton wool on a flexible copper wire 9" long and were fitted inside the glass test tubes with cotton wool plug. They were sterilised by autoclave under 15 lbs. pressure for 15 minutes.

Collection of Swabs :-

(a) Experiment No.: 1 - The external nares of healthy goats were sterilised by alcohol and the material within the nasal cavity was swabbed aseptically by rotating it 8-10 times. The goats number were noted for identification.

The materials were enriched immediately by inoculating into nutrient broth, and incubated aerobically at 37°C ± 1°C for 6-8 hours. After incubation, a loopful of the broth culture was inoculated on 8.0% bovine blood agar plates, and incubated aerobically at 37°C ± 1°C for 24 to 48 hours. Thereafter, the growth of organisms that appeared on blood agar plates was examined by recording the characters of colonies presence or absence of haemolysis, and chromogeneses etc.

The descrete colonies were picked up to study the morphology, biochemical and metabolic characters.

(b) Experiment No.2- Swabs were taken from the nasal cavity of the goats as used in experiment no. 1 but after experimental infection with Rinderpest F.D.G.T.V. virus. This was collected on the 5th day after infection just before they were slaughtered. At the time of slaughter, the goats were showing temperature 104°F to 107°F and were in state of viraemia .

(c) Experiment No.3 - The goats used in experiment no. 1 and 2 were slaughtered and the tracheal swabs were collected from these animals. The swabs from trachea were collected by introducing them into the upper part of trachea aseptically and rubbing it well into the middle and lower part of trachea as far as possible. The materials thus collected were processed in the same manner as described in experiment no. 1 & 2 .

Collection of material from lungs :- Portions of lungs from goats used in the above experiments, one piece from apparently normal area and other from pneumonic area of lungs were collected. They were cut into smaller pieces with sterile scissors. The materials were cultured on blood agar plates and subsequently studied as stated earlier .

Identification of Organisms :- All the isolates recovered from the swabs and lungs were identified following the methods of Bergy(1957) .

The criteria adopted for the identification were as follows.

STAPHYLOCOCCI

The organisms recovered on agar plates were examined for their colonial characters. Morphology was studied after staining the

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STAPHYLOCOCCI

The organisms recovered on agar plates were examined for their colonial characters. Morphology was studied after staining the

smears by Grams' method .

Catalase test :- The organisms were grown on agar plates over night. Drops of hydrogen peroxide were added on colonies. The production of gas bubbles was taken as positive for catalase enzyme.

Coagulase test :- The staphylococcal isolates were subjected to coagulase test. This was done as follows:

Mixed a loopful of the overnight grown culture from agar plate with 0.5 ml. of freshly diluted (1:10) rabbit plasma, incubated at 37°C and was then examined for the production of clot after 2 , 4 and 6 hours. If negative, the culture was left overnight at room temperature. Formation of fibrin clot indicated positive reaction.

Haemolysis :- For this, the organisms were grown on bovine blood agar plates aerobically at 37°C for 24 hours. The presence of wide zone of clear haemolysis and a narrow zone of incomplete haemolysis around the colonies was regarded as alpha and beta haemolysis respectively. A narrow zone of clear haemolysis was considered as delta haemolysis. All such strains were recorded as haemolytic. Those causing no alteration on blood agar plates were recorded as non-haemolytic.

Chromogenesis :- Pigment production by staphylococci was examined after 24 and 48 hours incubation at 37°C, then further after 24 hours at room temperature. The production of golden, white and yellow colours were noted.

ESCHERICHIA

Morphological studies of the organisms were done on young growing nutient broth culture after over night incubation at 37°C.

The smears were made and stained with Gram's method and examined. Motility was examined in hanging drop preparation.

For cultural characters, they were grown on Nutrient agar, blood agar and nutrient broth media, using conventional methods and colonial morphology, haemolysis, turbidity etc. were studied.

For biochemical studies, tests for Indol, M.R., V.P., Nitrate reduction, citrate utilisation and gelatin liquefaction, H_2S production etc. were done.

The fermentative activity of the isolates were done on lactose, sucrose, maltose, mannitol, inositol, glucose and dulcitol.

STREPTOCOCCI

For the study of the morphology of the organisms, they were grown as described above on bovine blood agar plates and smears stained by Gram's method examined .

For colony characters, the organisms were examined as described earlier. The haemolysis such as alpha(greenish discolouration) and beta(complete lysis of red cells) around the colonies were noted.

Catalase test was done as described for Staphylococci.

Sugar fermentation test was done using trehalose, salicin, mannitol, lactose, sorbitol, arabinose and inulin.

SALMONELLA

Morphological studies were done on young growing nutrient agar culture after overnight incubation at 37°C. The smears from the culture were stained by Gram's method and examined. Motility was checked in hanging drop preparation from broth culture .

For cultural characters, the strains were grown on nutrient agar, blood agar, nutrient broth; morphology, haemolysis, turbidity

etc. were studied according conventional procedures.

For biochemicals studies, tests for Indol, M.R., V.P., nitrate reduction, citrate utilisation, gelatin liquefaction and urease, and H_2S production etc. were done .

The fermentative activity of the isolates were tested in glucose, mannitol, maltose, serbitol, arabinose, trehalose, lactose, sucrose, salicin, adonitol, inositol, dulcitol and rhamnose.

The isolates which on biochemical and sugar fermentation reactions were identified as Salmonella were further checked with genus specific Salmonella " O - 1 " phage . Those which gave lysis were also tested against Salmonella Poly 'O' and Poly 'H' antisera by slide agglutination method.

PASTEURELLA

Morphological study was done on young growing broth culture after overnight incubation at 37°C. The smears from the culture were stained with Gram's and Leishman stain and examined. Motility was checked in hanging drop preparation from the broth culture.

For cultural characters, the strains were grown on nutrient agar, blood agar, nutrient broth and colonial morphology, haemolysis, turbidity etc. were studied using conventional procedure .

Biochemical tests as done for the other micro-organisms including Methylene Blue reduction test were performed .

For sugar fermentation test, glucose, maltose, mannitol, salicin, sucrose, dulcitol and arabinose were used.

The pathogenicity tests of the isolates were done by inoculating the organisms intreperitonesally and sub-cutaneously in rabbits and subcutaneously in guineapigs.

ASPERGILLUS

The films were examined unstained as well as after lightly staining with Gram's stain.

For cultural characters, Sabraud's agar media was used.

CHAPTER IV.

RESULTS

I. Studies of organisms isolated from the upper respiratory tract of calves :

As described in previous chapter(Material and Methods), nasal swabs from 82 apparently healthy calves were examined bacteriologically. From them, a total of 109 isolates were recovered. Based on systematic bacteriological examination, 4 were Past. multocida, 40 staphylococci, 9 E. coli, 5 streptococci and 51 were other aerobic gram-positive and gram-negative organisms. The result of isolation of different micro-organisms are described below :

PASTEURELLA

During the course of the study, Pasteurella organisms were isolated from the nasal cavity of 4 out of 82 healthy calves. Morphologically they were small gram-negative rods showing bipolar staining with Leishman's stain (Fig. I).

Cultural characters.

On agar plate, the colonies after 24 hours incubation at 37°C were round, low, convex, amorphous, smooth, glistening with entire edge. In broth culture, moderate growth with slight turbidity was evident in 18 hours. On blood agar plate, good growth was noticed with convex translucent colonies having smooth surface. There was no haemolysis on bovine blood agar plate. In gelatin medium, good filliform growth was observed but there was no liquefaction.

Biochemically, the strains were Indol positive, M.R.negative,

V.P.negative, Nitrate was reduced to nitrite, citrate was not utilised, Methylene Blue was reduced and Hydrogen sulphide was produced.

Among the carbohydrates, glucose, mannitol, and sucrose were attacked with the production of acid but no gas. Maltose, salicin, dulcitol and arabinose were not fermented. Based on above observations, they were identified as Past.multocida. The sugar fermentation and biochemical reactions are shown in table no. I .

Pathogenicity .

The pathogenicity of these trains was determined by parenteral inoculation in rabbit. 0.5 ml. of a 16 hours old broth culture was inoculated in a pair of rabbits intraperitoneally and subcutaneously. The former died at 18 hours and the latter at 28 hours after experimental inoculation.

Organisms with characteristic bipolar staining were found in the smears prepared from the heart blood of these rabbits and pure culture of Past.multocida was obtained from the heart blood, spleen, liver and lung (Fig. II).

Thus, of 82 healthy calves, Past.multocida were isolated from the nasal cavities of 4 of them . Besides, one strain of Past.multocida was to be described later, also isolated from the lung of one out of 132 slaughtered goats examined.

The percentage of recovery the Pasteurella multocida was 4.9% in calves.

STAPHYLOCOCCI

The organisms recovered on agar plates were examined in stained preparations for their morphological study. Those organisms

Table 1 : Biochemical and [illegible] reactions of Past.multocida isolated from nasal swabs of calves and goats .

Sl.No.	Morphology: Gram's staining	Bipolar rods	Motility	Haemolysis	Growth on MacC. Agar	Biochemical reactions: Indol	Hydrogen Sulphide	Nitrate reduction	M.R. test	V.P. test	Citrate utilisation	Gelatin liquefied	M.R. reduction	Urea hydrolysis	Sugar fermentation: Glucose	Maltose	Mannitol	Salicin	Sucrose	Dulcitol	Arabinose	Pathogenic for rabbits	Identification of the species
1	-	+	-	-	-	+	+	+	-	-	-	-	+	-	A	-	A	-	A	-	-	+	Past.multocida
2	-	+	-	-	-	+	+	+	-	-	-	-	+	-	A	-	A	-	A	-	-	+	P.multocida
3	-	+	-	-	-	+	+	+	-	-	-	-	+	-	A	-	A	-	A	-	-	.	P.multocida
4	-	+	-	-	-	+	+	+	-	-	-	-	+	-	A	A	A	-	A	-	-	.	P.multocida
5*	-	+	-	-	-	+	+	+	-	-	-	-	+	-	A	-	A	-	A	-	-	.	P.multocida

* One strain of Past.multocida was isolated from a goat.

A = Acid
+ = Positive
- = Negative
. = Not examined for pathogenicity

Figure (I) Past.multocida in smear from 24 hours old nutrient agar culture showing bipolar staining (X1000) .

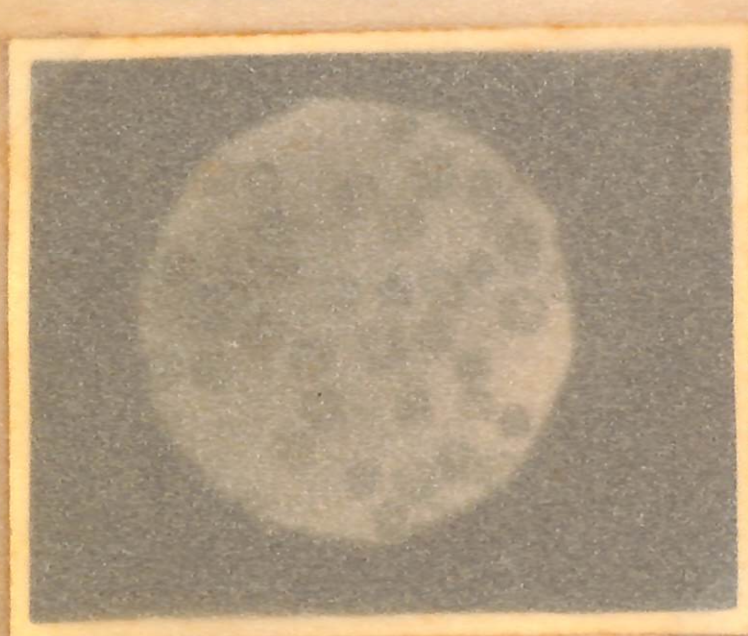

Figure (II) Past.multocida in . blood smear of experimental rabbit (X1000) .

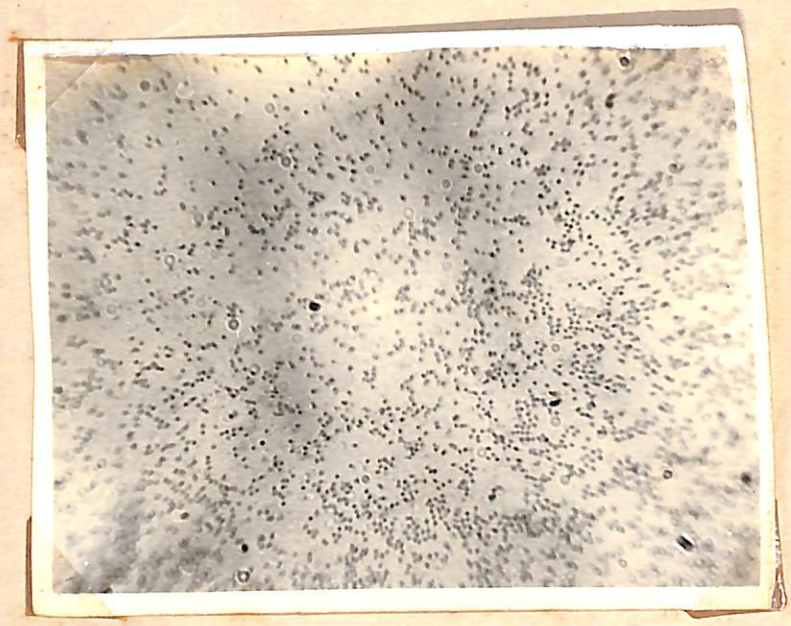

Figure (I) Past. multocida in smear from 24 hours old nutrient agar culture showing bipolar staining (X1000).

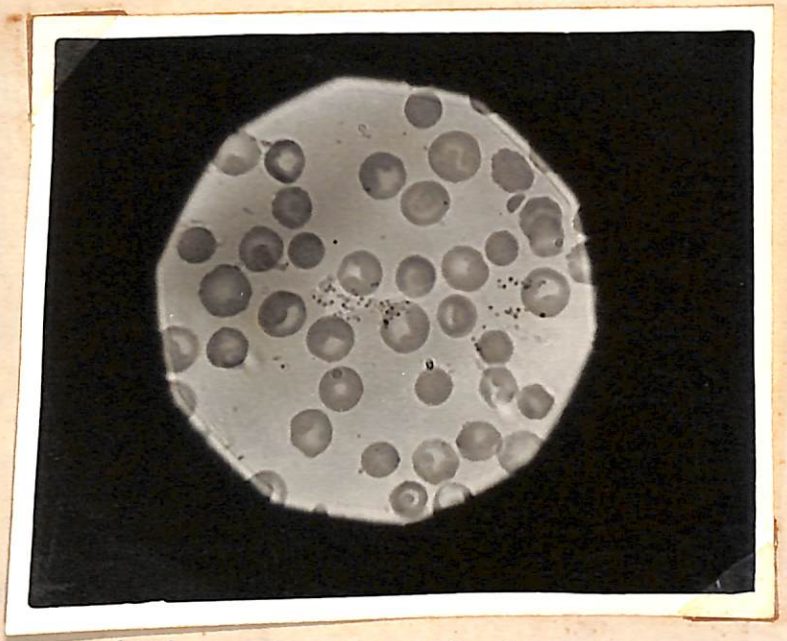

Figure (II) Past. multocida in a blood smear of experimental rabbit (X1000).

which were gram-positive and spherical in shape, and were arranged in clusters, and gave positive reaction on catalase and coagulase tests were regarded as staphylococci .

40 out of 109 cultures isolated from calves were Staphylococci. The organisms were isolated from nasal swabs of 40 of 82 apparently healthy calves. Staphylococci in pure culture were never isolated from any of the specimens. They were found always in association with other organisms. The percentage of the occurrence of these organisms against total number of isolates recovered and calves examined is presented in Table No. II .

TABLE NO. II

The incidence of Staphylococci in the upper respiratory tract of calves .

Organisms	No. of strains isolated	Percentage against total cultures(109)	Percentage in calves (82)
Staphylococci	40	36.64	48.8

Out of 40 strains of Staphylococci, 9 were Staph. aureus, 18 Staph. albus, and 13 were Staph. citreus.

Out of 40 strains of Staphylococci, 14 strains were coagulase-positive and 13 of these were haemolytic. The numbers of pathogenic and haemolytic strains of Staph. aureus, Staph. albus and Staph. citreus with their percentages are given in Table No. III .

TABLE NO. III

Showing the numbers, the percentage of coagulase positive and haemolysis of Staphylococci .

Isolates	Total no.	Coagulase positive		Haemolytic	
		No.	Percentage	No.	Percentage
Staph. aureus	9	9	100	9	100
Staph. albus	18	5	27.7	4	22.2
Staph. citreus	13	0	0	0	0

ESCHERICHIA ORGANISMS

The morphological appearance of colonies of Escherichia Organisms on agar plate(after 24 hours incubation at 37°C) were low convex and smooth. In broth cultures the growth was abundent showing uniform turbidity. On MacConkey's agar plates,smooth, convex and pink colonies were observed. The organisms were gram-negative and motile .

Biochemically, Indol was produced and Voge's Proskauer reaction (V.P.)negative. Nitrite was produced from nitrates,Methyle Red reaction (M.R.) was positive. Citrate was negative,gelatin was not liquefied, Hydrogen sulphide was not produced(except 2 strains) and Urea was not hydrolyzed .

Gas and acid were produced from lactose, maltose, mannitol, glucose,sucrose and salicin. Inositol was not attacked(except in 2 strains) and the reaction of dulcitol was variable.

Out of 109 cultures processed, 13 strains were found to be Escherichia Organisms, out of which 9 according sugar and biochemical tests were E. coli. and the rest were other species of Escherichia organisms. The percentage of the incidence of Escherichia organisms is given in Table No. IV.

TABLE NO. IV .

Percentage of incidence of Escherichia in calves.

Organisms	No. of strain	Percentage in cultures(109)	Percentage in calves(82)
E. coli.	9	8.2	10.9
Other species	4	3.6	4.8
Total	13	11.9%	15.8%

STREPTOCOCCI

The colonies of the Streptococci on blood agar plate were small, slightly raised circular and opaque. Fine granular deposits were observed in glucose broth culture. The organisms were spherical in shape and arranged in short chains and were gram-positive.

All strains were found catalase negative. They were beta haemolytic. Based on sugar fermentation reactions, they were identified as Streptococcus agalactiae, Strep. dysagalactiae and Str. zooepidemicus (Bergey's 1957).

From the nasal cavities of 82 apparently healthy calves, only 5 strains of streptococci were isolated. The percentage of incidence of streptococci in the total culture of 109 was 4.6% and the incidence in calves was 6.09% .

Out of 5 strains of streptococci recorded in the present study 2 strains of Str. agalactiae, one strain of Str. dysagalactiae and 2 strains of Str. zooepidemicus were identified on results of sugar fermentation reactions as shown below in Table V .

Table V sugar fermentation reaction of streptococci isolated from nasal swab of calves .

Sl. no.	Type of Haemolysis	Catalase	Sugar fermentation: Treh.	Salicin	Mann.	Lact.	Sor.	
1.	Beta.	-	A	A	-	A	-	Str. agalactiae .
2.	"	-	A	A	-	A	-	Str. agalactiae .
3.	"	-	-	A	-	A	A	Str. zooppidimicus .
4.	"	-	-	A	-	A	A	Str. zooepidimicus .
5.	"	-	A	-	-	A	A	Str. dysagalactiae .

A = Acid .

- = Negative .

OTHER ORGANISMS

Apart from organisms already described, some other organisms were also isolated from the nasal cavities of calves .

Pseudomonas : Out of 109 cultures, 4 strains were Pseudomonas. Morphologically the organisms were seen as gram-negative, small slender rods. The colony was large, and the spreading edges appeared after 18-24 hours incubation at 37°C on nutrient agar plate. The organisms were motile; greenish pigmentation was observed in broth culture .

The organisms were isolated from the nose of calves. Pure cultures were never isolated from any specimen examined. They were found always in association with other organisms .

The percentage of their incidence in relation to total cultures was 3.6% and in calves it was 4.8% .

Corynebacterium : Only two strains out of 109 cultures were identified as Corynebacterium organisms. The gram-positive pleomorphic rods were found in association with other organisms . The organisms were mostly slender with tapering end. They were non-motile, and non-spore bearing organisms .

On agar plate, the growth was very slow. The colonies 24 hours incubation at 37°C were small greyish-white in colour . On tellurite blood agar plate, the colonies were slaty black in colour .

The organisms were isolated from the nose of healthy calves. The percentage of the incidence in total culture was 1.8%, and in calves it was 2.4% .

Haemophilus :

One strains of this organism was found out of 109 cultures. Morphologically the organisms were seen as minute rods, pleomorphic, gram-negative, and non-motile .

Culturally the colonies on blood agar plate were tiny transparent and pin-point with smooth surface .

This was also found in association with other organisms . Pure culture was not isolated. They were isolated from the nasal cavity of a calf.

The percentage of the incidence of this organisms in the upper respiratory tract among 82 healthy calves was 1.2% .

Bacilli :

Gram-positive long spore bearing bacilli were isolated from the nares of healthy calves. In total, 30 strains (35.4%) were found in the nasal cavity of 30 of 82 calves. The percentage of the incidence in total culture is given in Table VI .

Table VI : The percentage of other micro-organisms in upper respiratory tracts of calves .

Sl. no.	Organisms.	No. of strains isolate.	Percentage against total culture(109)	Percentage against calves(82)
1.	Pseudomonas	4	3.6	4.6
2.	Corynebacterium	2	1.8	2.4
3.	Haemophilus	1	0.9	1.2
4.	Bacilli	30	27.3	35.3

Thus, out of 109 isolates recovered from the nasal swabs of 82 healthy calves, there were 4 Pasteurella, 9 E. coli, 40 Staphylococci, 5 Strentococci and 51 other organisms . No fungi were isolated from any of the specimen. The occurrence of different micro-organisms isolated is given in Table VII .

Table VII

Frequency of Isolation of Micro-organisms from the Nasal Cavity of 82 apparently healthy Calves.

Number of calves examined	Gram Negative Organisms						Gram Positive Organisms							Total number of cultures isolated
	B. coli	Pasteurella	Pseudomonas	Haemophilus	Other gram-ve rods	Total number of gram-ve organisms	Staphylococci	Streptococci	Micrococci	Corynebacterium	Bacilli	Yeast	Total number of gram-ve organisms	
82	9	4	4	1	10	28	42	5	4	2	30	0	81	109

II Studies of Organisms Isolated from the Respiratory Tract of Goats :

As reported earlier a total 132 goats were taken for study. From each goat, 5, meterials were examined i.e. nasal swabs one each before and after infection with C.T.V. virus and one each from trachea, pneumonic and normal lung .

From these goats a total of 660 isolated were recovered. They belonged to the groups staphylococci, streptococci, E. coli salmonella and others. The results of their isolation before and after experimental infection from different regions of respiratory tract are described below :

SALMONELLAE

The organisms were gram-ve rods occurring singly(Fig.III) and were motile. On agar plate (24 hours at 37°C), the colonies were circular, greyish in colour, and low convex with smooth surface and entire edge.(Fig.IV). In broth there was uniform turbidity .

All the strains were indol negative and produced hydrogen sulphide(H_2S); nitrite was produced from nitrate; they were M.R. positive and V.P. negative. Citrate was utilized, gelatin was not liquefied, and urea was not hydrolysed .

Acid and gas were produced from glucose, mannitol, maltose and sorbitol. None of the strains fermented lactose, sucrose, salicin, adonitol and inositol, but one strain attacked dulcitol .

Table IX : Presents the result of biochemical and sugar fermentation of Salmonella organisms.

Sl. No.	Morphology: Gram stain	Morphology: Rod shape	Morphology: Motility	Biochemical tests: Indol	Biochemical tests: Hydrogen Sulphide	Biochemical tests: Nitrate test	Biochemical tests: M.R. test	Biochemical tests: V.P. test	Biochemical tests: Citrate test	Biochemical tests: Gelatin liq.	Biochemical tests: Urea hydrolysis	Sugar fermentation: Glucose	Sugar fermentation: Mannitol	Sugar fermentation: Maltose	Sugar fermentation: Sorbitol	Sugar fermentation: Lactose	Sugar fermentation: Sucrose	Sugar fermentation: Salicin	Sugar fermentation: Adonital	Sugar fermentation: Inositol	Sugar fermentation: Dulcitol	Genus specific	Salmonella phase	Poly 'O' and Poly 'H' serum
1	-	+	+	-	+	+	+	-	+	-	-	AG	AG	AG	AG	-	-	-	-	-	-	+		+
2	-	+	+	-	+	+	+	-	+	-	-	AG	AG	AG	AG	-	-	-	-	-	-	+		+
3	-	+	+	-	+	+	+	-	+	-	-	AG	AG	AG	AG	-	-	-	-	-	AG	+		+
4	-	+	+	-	+	+	+	-	±	-	-	AG	AG	AG	AG	-	-	-	-	-	-	+		+
5.	-	+	+	-	+	+	+	-	+	-	-	AG	AG	AG	AG	-	-	-	-	-	-	+		+
6	-	+	+	-	+	+	+	-	±	-	-	AG	AG	AG	AG	-	-	-	-	-	-	+		+

+ = Positive .
- = Negative .
AG = Acid and gas .

Out of 660 isolates, 6 strains were provisionally typed as salmonella on the basis of biochemical and sugar fermentation reactions. These strains were also tested with salmonella genus specifi O-1 phage which showed clear lysis. These six salmonella strains were further confirmed by slide agglutination test using polyvalent 'O' and polyvalent 'H' antisera .

Thus out of 132 goats, 6 yielded salmonella organisms from pneumonic lungs . The nasal swabs, tracheal swabs and normal lung of these goats did not reveal any salmonella organisms. Thus, the percentage of salmonella infection causing pneumonia in goats was 4.5 %. This is shown in table X .

Table X . Occurrence of salmonella in pneumonic lung of goats.

No. of goats examined	No. of goat yielded salmonella	Percentage goat yielded salmonella	Percentage of isolated in total cultures
132	6	4.8	0.9

STAPHYLOCOCCI

Out of 660 isolates recovered from 132 goats, 192 strains of staphylococci were isolated from 71 goats. Of these, 85 coagulase positive strains recovered from 30 goats and the remaining 107 coagulase-negative strains were isolated from 41 goats. This is shown in Table XI .

Table XI . Occurrence of staphylococci in different part of respiratory tract of goats .

Organisms		Pre-infection	Post infection				Total
		Nose	Nose	Tracheal	Normal lung	Pneumonic lung	
Staph. Coagulase positive	No.	31	28	10	5	11	85
	Percentage	36.4	32.9	11.7	5.8	12.9	44.7
Staph. Coagulase negative	No.	40	43	11	6	7	107
	Percentage	37.7	40.8	10.2	5.6	6.5	55.3

Out of 192 strains of staphylococci, 54 were golden in colour, 89 were white and 49 were yellow pigmented. The number of coagulase-positive strains among golden and white staphylococci were 54 and 31 respectively. Further all golden strains were haemolytic, but only 24 out of 89 white strains were haemolytic. The remaining 7 white coagulase-positive strains were non-haemolytic. The relationship between haemolytic and coagulase activity and chromogenesis is presented in table XII .

Table XII . Relationship between coagulase and haemolytic activity and chromogenesis of staphylococci from respiratory tract of goats .

Pigment	No. of strains	Coagulase positive		Haemolytic	
		No.	%	No.	%
Golden	54	54	100	54	100
White	89	31	34.6	24	26.9
Yellow	49	0	0	0	0
Total	192	85	44.2	78	40.6

Besides, gram-positive Diplococci and Micrococci(Coagulase- and catalase-negative) were also isolated in association with other organisms as presented in table XIII .

Table XIII .Occurrence of gram-positive Diplococci and Micrococci in the respiratory tract of goat.

Organisms	Pre-infection	Post-Infection				Total
	Nose	Nose	Trachea	Normal lung	Pneumonic lung	
Diplococci	6	6	5	1	5	23
Micrococci	24	25	8	0	9	66

ESCHERICHIA

The morphological appearance of colonies of Escherichia organisms on agar plate (after 24 hours incubation at 37°C) was low convex and smooth. In Broth(24 hours old), the growth was abundant showing uniform turbidity. On MacConkey's agar plates, smooth convex pink colonies were observed. The organisms were gram-ve rods and motile . Biochemically, Indol was produced, Voge's Paskeur reaction (V.P.) was negative. Nitrite was produced from nitrate and Methyl red (M.R.) reaction was positive. Hydrogen sulphide was not produced (except in E. Freundii). Citrate was negative (except in E. intermedia) and gelatin was liquefied.

Gas and acid were produced from glucose maltose,mannitol, lactose sucrose and salicine .

Out of 660 total isolates, 53 belonged to Genus Escherichia. They were recovered from nasal and tracheal swab and from lungs of 30 out of 132 goats examined .

Out of 53 strains, 41 strains were typed as E.coli 7 strains were identified as E. freundii and 5 strains were identified as E.intermedia .

The percentage of occurrence Escherichia organisms has been presented in table XIV .

Table XIV . Occurrence of Escherichia in respiratory tract of goat before and after infection.

Organisms	Pre-infection	Post infection				Total
	Nose	Nose	Trachea	Normal lung	Pneumonic lung	
E. coli	13	13	5	1	9	41
E. intermedia	1	1	1	0	2	5
E. freundii	2	3	0	0	2	7

STREPTOCOCCI

The colonies of the streptococci on blood agar plates were small, slightly raised circular and opaque. Fine granular deposits were observed in glucose broth culture . The organisms were spherical in shape and arranged in short chains and were grame-positive .

All the strains were found to be catalase-negative. They were beta haemolytic except one which was alpha haemolytic. The latter was observed as partial greenish discolouration on blood agar plate .

Acid was produced from lactose and sorbitol, but not from mannitol and salicin (Str. dysagalactiae). Acid was produced from trehalose, salicin, lactose, mannitol but not from sorbital (Str. agalactiae) . Acid were produced from salicin, lactose,

and sorbitol but trehalose and mannitol were not fermented (Str. zooepidemicus) .

Based on sugar fermentation reaction , they were identified as Str. agalactiae , Str. dysagalactiae, & Str. zooepidemicus(Bergey, 1957) . Serological typing could not be done due to non-availability of antisera .

Table XV. Showing the differentiation of streptococci organisms .

Organisms	Sugar fermentation				
	Treh.	Salic.	Manni.	Lactose	Sorbitol
Str. agalactiae	A	A	-	A	-
Str. dysagalactiae	A	-	-	A	A
Str. zooepidimicus	-	A	-	A	A

Thus out of 660 isolates recovered from 132 goats; of 10 goats yielded streptococci. Of these 18 strains, 6 strains were from nasal cavity of normal goats, and infected goats nasal cavities yielded 3 strains, trachea 3 strains, normal lung, pneumonic lung 6 strains. The percentage of occurrence is shown in table XVI .

Table XVI .Occurrence of streptococci in respiratory tract of goats before and after infection .

Name of organisms	Pre-infection	Post infection				Total	Percentage
	Nose	Nose	Trachea	Normal lung	Pneumonic lung.		
Str. agalactiae	1	1	1	0	3	6	33.3
Str. dysagalactiae	3	2	1	0	1	7	38.8
Str. zooepidemic	2	0	1	0	2	5	27.9
Total	6	3	3	0	6	18	

OTHER ORGANISMS

Apart from organisms already described, other micro-organisms were also isolated from the respiratory tracts of goats .

Pseudomonas : Out of 660 specimens from 132 goats, 43 strains of Pseudomonas were isolated . They were Gram-negative small slender rods. The colonies were large with spreading edges after 18-24 hours of incubation at 37°C on nutrient agar plate. They were motile and produced greenish pigmentation in broth culture. The organisms were isolated from nasal swabs, trachea and lungs of the goats .

Corynebacterium organisms : Out of 660 culture, only 4 strains belonged to Corynebacterium. These organisms were found to be gram-positive, pleomorphic rods with tapering ends. They were none motile, non-sporing organisms .

On agar plate, the growth was poor. The colonies after 24 hours incubation at 37°C were small, greyish-white in colour. On blood agar plate , the growth was better . On blood tellurite agar plates, they produced small black colonies . Out of 4 strains of the Corynebacterium, 2 were recovered from the nasal mucosa and 2 from pneumonic lungs .

Proteus : Out of 660 cultures, only one strain was identified as Proteus organisms. They were isolated from the nasal swab of healthy goat .

Microscopicaly organisms were gram-negative rods, were arranged singly and in pairs end to end. Some showed short chain and long felementous forms .

On agar plate, the growth showed characteristic swarming characteristics. The growth was spread over the whole surface of

the agar plate and produced a thin uniform layer after 24 hours incubation at 37°C .

Bacilli : On cultural examination, the colonies were large having rough surface and irregular shape. They were isolated along with the other organisms from all parts of respiratory tract. Morphologically, they were gram-positive long spore-bearing bacilli .

Out of 660 cultures, 215(32.5%) cultures of bacilli were isolated . The percentage of recovery of these organisms is presented in table XVII .

Table XVII. Occurrence of other organisms in respiratory tract of 132 goats .

Organisms	Pre-infection	Post infection				Total	Percentage
	Nose	Nose	Trachea	Normal lung	Pneumonic lung		
Pseudomonas	12	12	7	1	11	43	6.2
Corynebacterium	1	1	0	0	2	4	0.6
Proteus	1	0	0	0	1	1	0.15
Bacilli	68	57	51	8	31	215	3.5

In summary, out of 660 isolates recovered from the upper and lower respiratory tract of 132 goats, there were 192. Strains of staphylococci, 89 other micrococci, 53 Escherichia organisms, 18 streptococci 6 salmonella and 301 other organisms.

The occurrence of different micro-organisms isolated is given in table XVIII . A comparison between the percentage of isolation between calves and goats is presented in Figure V.

Table XVIII. Frequency of Isolation of Micro-organisms from the Respiratory Tract of 132 Goats.

Source		Gram-negative organisms							Gram-positive organisms							Total	Percentage
		Salmonella	Pasteurella	Escherichia	Pseudomonas	Proteus	Unidentified organisms	Total no. of G-ve organisms	Staphylococci	Streptococci	Micrococci	Corynebacterium	Bacilli	Fungi	Total No. of G+ve organisms		
Pre-infection	Nose	0	0	16	12	1	9	38	74	6	30	1	68	7	186	224	33.9
Post infection	Nose	0	0	17	12	0	9	38	68	3	31	1	57	7	167	205	31.1
	Trachea	0	0	6	7	0	2	15	21	3	13	0	51	1	89	104	15.7
	Normal lung	0	0	1	1	0	0	2	11	0	1	0	8	0	20	22	3.4
	Pneumonic lung	6	1	13	11	0	2	33	18	6	14	2	31	1	72	105	15.9
	Total	6	1	53	43	1	22	126	192	18	89	4	215	16	534	660	100.00

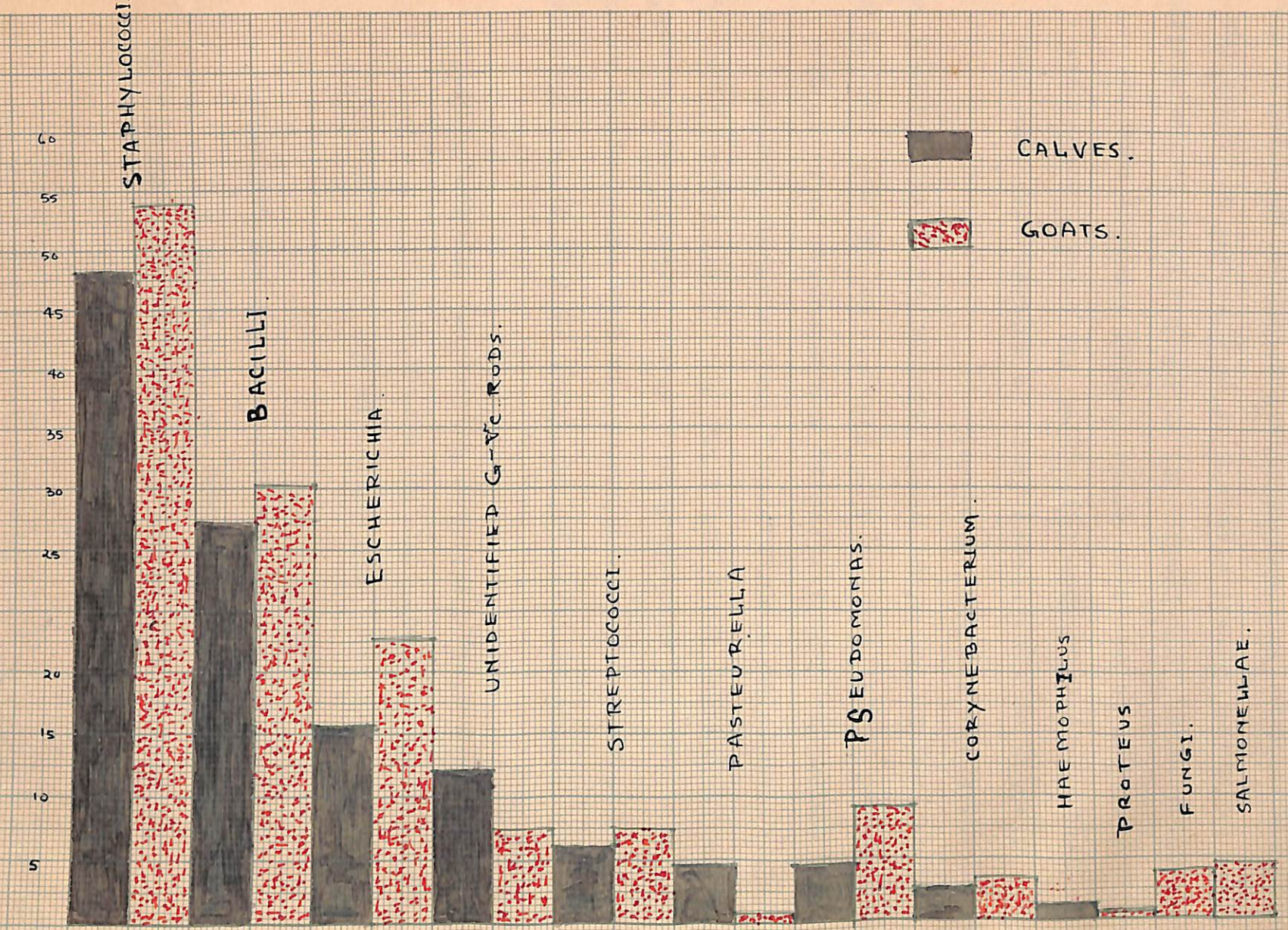

FIG: V.

HISTOGRAM showing percentage incidence of different Organisms in Calves and Goats.

CHAPTER V :

DISCUSSION

The respiratory tracts in mammals may be divided into the following portions (Wilson and Miles,1966) .

1. Upper part including the anterior and posterior nares and nasopharynx .

2. Intermediate zone common to the respiratory and alimentary tract including the oropharynx .

3. Lower part, including the larynx, trachea, bronchi and lungs .

The bacterial flora of the nasal passage differs from that of naso-pharynx . In the former it is less numerous. If swabs from the nose and naso-pharynx of normal animals are examined bacteriologically, the nasal swabs give colony counts lower than those obtained in naso-pharynx . The difference observed are often striking. Quantitatively, staphylococci both (Staph. aureus and albus) are far more frequent in the nose than in the nasopharynx; on the other hand, non-haemolytic streptococci and micrococci are far less frequent.

Studies on respiratory flora in animals have been few and fragmentary. In some instances, the carrier rates have been assessed in relation to particular disease, such as pasteurellosis(Singh 1943). But systematic data on the bacterial flora in the different sigments of respiratory tract are far from complete . The results of investigation obtained in the present of study are discussed below .

PASTEURELLA

In the present study 4 strains of Past. multocida were isolated from the nasal swabs of 82 apparently normal calves within 3 to 6 months of age. The percentage of recovery of the organisms in them was 4.9%. Pathogenicity done with two of them in rabbits proved unequivocally that they were highly virulent. All the calves remained well till date in a very good state of health except one which died of bronchopneumonia 12 days after the collection of materials. Unfortunately, the the autopsy materials from this animal were not available for examination which could have revealed the pathogenicity of the organism in the natural host. The fact that the remaining three calves carrying Past. multocida in their nose for a long time in the same pen kept in the same environment without causing any apparent illness suggests that there are certain unknown host factors which determine the outcome of such infection.

This finds elucidation from the observation of Prasad(1962) who isolated Past. multocida from spleen of a dead R.I.R. hen of an organised poultry flock. He considered the strain to be an atypical virulent on the basis of pathogenicity test in different susceptible experimental hosts. About five months after this isolation, a severe outbreak of fowl cholera occurred in the flock. The author felt that strains of Past. multucida exist in a flock which may under certain circumstances remain deficient in the some of the biological attributes of classical Past. multocida or it may well be that the deficient strains become pathogenic during natural passages through the susceptible host in the flock.

The persistance of Past. multocida in the respiratory tract of natural hosts has been reported by a few workers in the past. Singh(1948) isolated 3.5% of this organism from live cattle and 7% from dead cattle. Bain(1961) observed that the carrier rate of the Past.multocida in Asian countries was 5%, while Kumar in 1965 reported its occurrence in normal calves as 2.2% . The present investigation has revealed the carrier rate of Past.multocida in healthy calves very close to that of Bain(loc.cit). Due to non-availability of known antisera, the serotype of the strains recovered could not be determined. This could have disclosed the pathogenic potential of the organism, since cattle are known to be susceptible to a particular serotype- Robert type I (Bain 1954).

Out of 132 goats examined, only one goat yielded Past. multocida from pneumonic lung showing the carrier rate in this species to be 0.7% . The serotype of the strain was could not be determined. Informations regarding the carrier rate in Indian goats are only few and far between. While in normal sheep, it was found to vary between 5.6% (Omar et al,1962) and 12.5% (Nandita & Rao, 1969), its percentage in goats was reported to be 0 (Ramchandran & Sharma,1969). Pasteurella are known to be one of the common etiological agents of pneumonia in sheep and goats(Newsom and Cross 1932). However, the informations about the carrier rate of Past.multocida in goats is very sketchy . The serotype of Past.multocida causing pasteurellosis in goat is reported to be Robert type 1 or carter type B(Nikiphorova 1958) . It is not known if or to what

extent the carrier goats are responsible for pasteurellosis in cattle.

STAPHYLOCOCCI

In the present study, staphylococci were isolated from the nose of 40(43.3%) out 92 calves examined. Of 40 strains, 14(35%) were coagulase-positive and 26 65% were coagulase-negative. The incidence of staphylococci estimated with reference to the total number of isolates from calves was 109(31.6%). This is consistent with findings of other workers(Kumar,1965).

Similarly, out of 132 goats examined, 71(53.8%) yielded staphylococci. Of these, 30(42.2%) carried coagulase-positive staphylococci, and the remaining 41(57.8%) had coagulase-negative strains. The percentage of recovery of staphylococci in goats in the present investigation was found to be slightly less than reported by Sowhney(1959) who found it to be 62%.

A detailed study to determine the characters of pathogenicity of the strains was not done. Those that were coagulase- and catalase-positive were regarded as Staphylococci . From table III, it will appear that all the golden strains from calves were coagulase-positive as well as haemolytic, where as among the white strains only 27.7% were coagulase-positive and 22.8% were haemolytic. None of the yellow-pigmented strains showed either coagulase-or haemolytic-activity. Similar results were obtained from strains isolated from goats.

Coagulase activity has been found under certain circumstances to be an unstable character(Nag and Prasad,1965). Further, coagulase-negative strains have been found to cause endocarditis in man (Resnekov,1959) although one characteristic alone can not be relied upon too much to screen the incidence of pathogenic Staph. aureus(Baird-Parker,1965), it seems that for all practical purposes, coagulase test can be profitably used in such survey of the microflora in animals and man .

From table XI, it can be seen that the percentage recovery of coagulase-positive staphylococci in the nose of goats before & after infection was not significantly different. In the lower respiratory tract, their numbers were far less frequent although in pneumonic lungs the percentage was two fold greater than in normal lung. The percentage of coagulase - negative strains under such conditions remained practically the same. It seems that in cases of inter-current infection or when host's resistance is lowered due to other factors, these resident staphylococci overcome the host's defence barrier and invade the system. A similar situation has been reported to exist in other infections(Prasad and Ahmed,1965) .

The study of nasal carrier state, colonisation and transmission provides many intriguing problems regarding the pathogenesis of staphylococcal infection in animals and man. Some remain persistent carriers but others do so only intermittently. A few others may defy all attempts by staphylococci to become established in nasal mucosa. This suggests that apart from the difference in the infectivity of the strains, there

are some intrinsic responses of the host which permit or prevent establishment of infection. In human babies, the implantation of inocuous strains of staphylococci in the umbilicus stump or in the nose has been found to interfere in the outbreaks of staphylococcal infection in new-born infants. (Blair,1965). It seems reasonable to assume that the outcome of any infection depends upon a combination of factors existent in the parasite as well as the host .

ESCHERICHIA

Out of 109 culture obtained from the nasal swabs of 82 healthy calves 13 strains of Escherichia organisms were isolated from 13 calves . This showed the carrier rates of 15.8% . Kumar(1965) isolated 22.5% Escherichia organisms from upper respiratory tract of healthy calves. Singh(1965) isolated Bacterium coli in association with other bacterial flora in throat in sick dogs without showing respiratory infection.

Similarly out of 600 isolates recovered from 132 goats, 53(8%) strains of Escherichia organisms were isolated from 30 goats (22.6%). Dubin et al(1943) reviewed the cases of pneumonia associated with Bacterium coli infection in man. They studied the route by which the organisms reach the lungs in man. They considered that the most likely route was by aspiration. The other possibility was the transference of the organisms from gastro-intestinal tract .

Pelehev et al(1967) studied the aetiology of enteritis in newborn calves in Bulgaria. They examined a total of 353 specimens obtained from calves, 1-10 days of age at 20 farms.

They isolated pure culture of E.coli from the nasal mucosa of 80% of calves.

Out of 53 Escherichia species, 16 strains were recovered from the nasal cavity of healthy goats; the rest isolated post-infection were 17 from nose, 6 from trachea, 1 from normal lung and 13 from pneumonic lungs . Out of 132 C.P.V. infected goats, 13(17.5%) were associated with, apart from other organisms, Escherichia . As set out in table XIV, it will appear that 53 strains belonging to 3 species of Escherichia were isolated from goats in which 41(77.3%) were E.coli . This predominated over E.intermedia (5 strains) and E. freundii(7 strains). Of 41 strains of E.coli , 13 were isolated from nose both pre-and post-infection with P.P.C.P.V.virus. In trachea and normal lung, their numbers were 5 and 1 respectively, but in pneumonic lung, the number rose to 9. This suggests that under the devitalising conditions resulting from viraemia, the organisms invaded lung perhaps through the haematogenous route and not through the air passage (bronchial ramifications).

Pneumonia due to E.coli is common in animal and in man. Many workers isolated E.coli from pneumonic-lungs. This organism is the causative agent of the chronic respiratory infection in animals, humans and poults. The transition of E.coli from the gastro-infestinal to the respiratory tract is common. These have been reported to be transmitted by blood strain(Gross 1958).

It is fairly known that E.coli remain for long as saprophytes in soil and environments. These constitute the

potential source of infection to young animals which are more susceptible than the older stock. A similar situation exists in human babies and children. Once they become established in intestinal tract, the hosts remain as persistent or intermitter carriers. Thus, they cause, under different interisic or environmental factors, a variety of pathological conditions depending upon their pathogenicity, serotypes and host resistance

STREPTOCOCCI

5(6%) out of 82 healthy calves revealed streptococci in their nose. Among 132 goats, 10(7.5%) were found to carry streptococci in their respiratory tract. A total of 18 strains were recovered from different parts of the air passage. Six strains were found in their nose pre-infection, but only 3 post-infection. After infection, 3 showed up intrachea, 0 in normal but 6 in pneumonic lung. All the strains isolated from goats were beta haemolytic except one which was alpha haemolytic. The percentage of recovery of streptococci in calves and goats are similar to Kumar(1965) and Ramchandran & Sharma(1969).

Numerous observers have demonstrated the presence of haemolytic streptococci in the dust of wards or dormitories housing human patients, or crowded bars of animals suffering from respiratory infection due to streptococci. They have attached considerable significance to aerial spread by contaminated dust. There is increasing evidence to incriminate the nose as the principal reservior of infections since it is from the nose that the large infective droplets are most likely to come

Streptococci play part in causing bronchitis and primary pneumonia in animal and human being. In chronic bronchitis that causes so much illness and death in the animals, streptococci are almost always present in the bronchi(Burrow,1959).

Septicaemia with sudden death in calves has been recorded in which streptococci were the apperent cause.(Donald and Mann 1950). Str. zooepidemicus has also caused heavy losses in sheep with up to 90% mortality occuring in group of lambs(Nafyi and Mir Chemsy 1953). Pneumonia in calves may be caused commonly by Str. pneumoniae in some cases and unidentified steptococci have been ascribed as common invaders in viral pneumonia of calves(Hammer 1956). Since in the present investigation, the percentage of isolation of streptococci in the respiratory tract of goats following infection was reduced considerably except in pneumonic lung which compared well with the nasal swab pre-infection(Table XVI), it is reasonable to assume that the pathogenesis of streptococci associated with viral pneumonia in goats is in some cases similar to that contended for E. coli .

SALMONELLA

Out of 132 goats examined, six showed Salmonella organisms in their lung . In all these cases, the lung showed varying degree of hepatisation. Thus, the incidence of salmonella infection in G.T.V.infected goats was 4.5% . None of the materials from nose, trachea and normal lung of these goats, or the nasal swabs of 82 healthy calves yielded salmonellae.

There is no reason to doubt that the members of the salmonella group are primarily intestinal parasites, though they may also be isolated from blood and internal organs such as lung, heart, liver, kidney and gallbladder and etc .

Salm. typhi-murium and Salm.enteritidis cause infections of rats and mice and these animals become healthy carriers of the bacilli(Edward et al 1943).

Contrary to what had been previously supposed, the dogs may be ifected with salmonella up to the extent of 15% .While it seems unlikely that the dog is an important reservoir of human infection, it has been found to transmit in-fection to man. (Burrows 1952) .

In goat, salmonella infection has a rather localized distribution. There have been numerous outbreak of gastro-enteritis in sheep due to Salm.typhimurium in New Zealand and Australia(Josland 1950; Watts and Wall 1952). Salmonella dublin may cause abortion or diarrhoea both in goats and sheep(Levi,1949, Shearer 1957 and Watson 1960) .

Cattle, sheep, goats, rodents and poultry act as carrier or temporary excretors of salmonella organisms , their primary site of residence being the alimentary system. In contaminated environments, they may persist for a varying period of time depending upon the ecological factors. It is not improbable that Salmonella may gain entry into the nose of calves and goats from the contaminated environments. Nevertheless, failure to find salmonella in the respiratory tract of calves and goats except

from the hepatised lung of the latter suggests that these organisms normally do not proliferate or persist in respiratory tract except when the defence of host is weakened by various stress factors and intercurrent infection. Under such circumstances, these organisms invade lung from the intestinal tract and cause pneumonia .

OTHER ORGANISMS

As has been stated previously, a large varieties of organisms belonging to different genera were isolated from the same material. None of them was isolated in pure culture. Apart from organisms described earlier, many others were si isolated among which the bacilli predominated both in calves and and goats . The next in order of frequency was the pseudomonas (Table VI &XVII) . Others were less frequently encountered. The results of isolation of different microorganisms from the respiratory tract of calves and goats are summarised in Table VII & XVIII respectively. It is not clearly known whether or to what extent colonisation of nose by the dominant microflora such as the bacilli interfere with the multiplication of other organisms. It is, however, not unlikely since the Bacilli are known to elaborate antibiotics and inhibitory substances . Some of these biological intrigues, which may determine the bacterial flora and the pathogenesis of respiratory infection in animals and man remain to be resolved .

CHAPTER VI :

SUMMARY

In the present study, an attempt was made to determine the aerobic bacterial flora of the respiratory tract of young calves and goats .

Nasal swabs from apparently healthy calves and goats, and swabs from the nose, trachea and lung of these goats after experimental infection with F. M. D. V. virus were examined bacteriologically. The convectional methods for the identification of the isolates were followed .

In 82 calves, staphylococci predominated over all the other nasal flora. The lowest percentage of recovery was that of Haemophilus. Non of the organisms was isolated in pure culture . The percentages of isolation of different organisms from calves in order of frequency were as follows :

Staphylococci	43.8 % .
Bacilli	27.3 % .
Escherichia	15.8% .
Unidentified(Gram-ve)	12.1 % .
Streptococci	6.1 % .
Past. multocida	4.9 % .
Pseudomonas	4.8 % .
Corynebacterium	2.8 % .
Haemophilus	1.2 % .

The percentages of recovery of different organisms from goats were as under :

Staphylococci	53.7%.
Bacilli	22.7%.
Escherichia	22.6%.
Unidentified(Gram-ve rods)	7.5%.
Streptococci	7.5%.
Past. multocida	0.7 %.
Pseudomonas	9.1 %.
Corynebacterium	3.0 %.
Proteus	0.7 %.
Fungi	3.0%.
Salmonella	4.5%.

Four pathogenic strains of Past. multocida were isolated from the nasal swab of 32 healthy calves, the carrier rate in them being 4.9%. On the other hand, [redacted] none of 132 healthy goats was found to carry Past. multocida in their nose. Only 1(0.7%) of them yielded this organism in the lung specimen which was baptised. The data suggested that certain intrinsic host factors determine the outcome of such infection.

In calves, the percentage of coagulase-positive strains was 35 against 42.2 found in goats. All the golden strains of Staph. aureus were coagulase-positive and haemolytic but the reverse was not true. None of the yellow strains was either coagulase-positive or haemolytic. Evidences were brought out to show that the resident staphylococci present in nose invade lung when intercurrent virus infection or other stresses are

present. Thus, the outcome of staphylococcal infection depends upon a combination of factors existent in the parasite and the host.

Among 32 calves, 15.8% were nasal carrier of Escherichia organisms in contrast to 22.6% found in goats. E. coli predominated (77.3%) over other species. The data suggested that under devitalising conditions resulting from viraemia, E. coli reach lung through haematogenous route and not through air passage. The pathogenesis of the disease is discussed.

About 6% of calves were found to carry haemolytic streptococci against 7.5% in goats. It was contended that apart from droplet infection, pathogenesis of streptococci associated with viral pneumonia in goats was similar to that of E. coli.

Salmonella organisms were isolated from 4.5% of hepatised lung of 132 goats infected with P.P.G.E.V. Virus. The evidence suggested that salmonellae invade lung from the intestinal tract and that they do not proliferate or persist in respiratory tract except when the hosts' defence is weakened by intercurrent infection or other stress factors.

In the respiratory tract of calves and goats, staphylococci showed the highest frequency of isolation. Bacilli predominated over all other micro-organisms. It was contended that perhaps some inhibitory substances elaborated by bacilli interfere with the multiplication of some of the bacterial flora in the respiratory tract of cattle goats. Further studies on some of these biological intrigues which may determine the pathogenesis of respiratory infection in animals and man was suggested.

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