Bacterial Flora including *Mycobacteria* of the Respiratory Tract of Cattle and Buffaloes in Bihar

A THESIS

Submitted to the Faculty of Veterinary Science

Rajendra Agricultural University, Bihar

in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE (VETERINARY)

1971

Post-Graduate Department of
BACTERIOLOGY
BIHAR VETERINARY COLLEGE
PATNA

By

MD. SABIR ANJUM

G. B. V. C., B. V. Sc. & A. H.

R. A. U. Junior Research Fellow

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I certify that Dr. Md. Sabir Anjum has worked under my guidance and supervision on the problem of "Bacterial Flora including Mycobacteria of the Respiratory Tract of Cattle and Buffaloes in Bihar " and has submitted this thesis in partial fulfilment of the requirements for the degree of Master of Science (Veterinary) in Bacteriology of the Rajendra Agricultural University, Bihar. It incorporates the results of his independent research done under my guidance.

T. S. Sharma
25.3.72.

March 25, 1972.

(T.S. SHARMA)

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In the memory of my
mother and mother-in-law who lost
their lives by accidental burn.

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I N T R O D U C T I O N

Animals are exposed to a variety of microorganisms through out the span of their life right up from the day they are born. Microbe colonisation begins at birth, the first settlers being transferred from the maternal vagina and perineal areas to the calf skin, mouth, nose and orophrynx. The characteristic microflora of these and other areas develop latter and are probably derived and selected from various environmental sources.

After gaining entry into the host true saprophytes fail to maintain themselves but virulent organisms may survive and set up disease under suitable conditions in the hot they invade.

The chief portals of entry and infection are the digestive, respiratory, urinogenital tract and skin. Of the different portals of entry, respiratory tract proves to be the most vulnerable.

Both primary and secondary infections of the respiratory system are common and mostly due to inhalation of infective droplets and droplet nuclei. Generally, these primary and secondary infections are associated with bacteria, viruses, fungi and rickettsiae.

Microbes are present in inspired air but are rapidly removed during passage through the tortuous nasal apparatus lined with mucous memberane to whose moist surface they cling. Majority of the inhaled bacteria are arrested into upper respiratory tract but a few gains entrance through bronchii into the

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bronchioles. The process is so efficient that only very few of the organisms are thrown back after lodgement in inspired air except those that are expelled out in droplets by sneezinging and coughing. Thus several diseases are conveyed in air born infection. (Burrow,1959) The organisms that penetrate the upper respiratory passage are deposited in bronchii and bronchioles and are probably phagocytosed. When the equilibrium between the host and the invading organisms is disturbed due to several environ-mental factors, the microorganism gains upper hand and sets up primary and secondary infections.

Several workers have tried to pick out the microbial agents that are associated with the respiratory passages and their roles in setting up respiratory troubles in cattle and buffaloes.

Of different microorgnisms that are commonly associated with respiratory infections,most frequently found to cause disease in cattle and other domesticated animals are Staphylococcus, Corynebacterum, Pseudomonas, Proteus, Haemophilus,Klebsiella and Salmonella. Mycoplasma have been incriminated from pneumonic lungs of calves. (Davis 1967, Gourlay 1969 and Leach 1970.).

The vulnerability of the respiratory tract to infection in an animal and its importance in transmission of the disease to other animals and man is evident from the foregoing accounts. Taking into consideration the importance of the respiratory tract this study was undertaken to find out the serobic microflora present in the different parts of the respiratory tract of cattle, buffaloes and calves.

This study, I trust, will assist in coming to a better

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understanding of the pathoganesis and epidemiology of respiratory diseases of domesticated animals from whom the microorganisms have been isolated and identified.

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REVIEW OF LITERATURE

The Genus Pasteurella.

Mobb (1933) recovered Pasteurella bovis from the pneumoni -c lungs of animals at Mukhteshwar (U.P.).

Newson and Gross (1932) recovered Pasteurella organisms from cattle and sheep suffering from pneumonia. Schenk (1938) isolated Pasteurella microorganisms from air passages of cats.

Bythell (1945) described two forms of Pasteurella infection in bovine. In the peracute form which was the septicaemic form, rapid death of young calves was noticed (Haemorrhagic septicaemia). The other form was bronchopneumonia. Pasteurella organisms were detected in both the cases.

Singh (1948) recovered 3.5 percentage(%) of Pasteurella. septica from the live and 7.0 % from the dead cattle. The above organisms were recovered from the nasal cavities of young calves.

Carter (1958) isolated Pasteurella. multocida from the affected lungs of calves. Hamdy et al (1959) recovered Pasteurella septica and Pasteurella. haemolytica from pneumonic lesions in lambs.

Hoerlein et al (1961) recovered a large number of Pasteurella organisms from the lungs of cattle showing bronchopneumonia. Affected animals showed more Pasteurella in their nasal mucosa than did the normal animals.

Collier et al (1962) described that cattle of different age groups may be infected by Pasteurella. haemolytica and Pasteurella multocida causing pneumonia but animals between six months and two years of age were more susceptible than young

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calves and adults.

Omer et al (1962) recovered a virulent strain of pasteurella. multocida from the nasal cavity of an apparently healthy buffalo.

Schipper (1963) studied pneumonia caused by pasteurella. This disease was more common in cattle in Europe, U.K. and America but not in Australia. The morbidity and mortality in young beef cattle caused by Pasteurella. were recorded to be 17.0 % and 7.5 % respectively. Kumar (1965) recovered 2.2% of Pasteurella. multocida from the nasopharynx secretion of 135 apparently healthy calves. Wigewanta and Kurumaratne (1968) studied the occurrence of Pasteurella.multocida in the nasopharynx of healthy cattle. Out of 300 cattle slaughtered at an abbatoir in Ceylone during a period of six months, he isolated Pasteurella multocida from 15% of cattle. All 45 strains isolated were pathogenic for mice and of these fortythree strains were Asian type I of Robert while two were identical to Australian type I.

Singh and Mallick (1968) isolated Pasteurella species from the respiratory tract of 8.5 percent of buffoloes slaughtered in an abbatoir.

Mohan et al, (1968) studied the immunity against Pasteurella. multocida in buff calves and their carrier status. A total of 205 buff calves screened were in the age group of one to one and one-half years. They had neither received any prophylactics against Pasteurella nor had the clinical disease. Nasal swabs from upper respiratory tract were examined. Only one calf yielded Pasteurella.multocida and this was avirulant for rabbit

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and mice. Serologically the isolate differed from the P52 strain of the Indian Veterinary Research Institute (I.V.R.I.) Mukhteshwar- Kumaon (U.P.).

Weisman & Hilkar (1968) studied the morphological, cultural, biochemical and serological characteristics of fortythree strains of Pasteurella haemolytica isolated from respiratory tracts of cattle. This included 37 cattle which had shipping fever. With one exception all strains satisfied the major criteria for differenciation of the species from Pasteurella. multocida. In fermentation reaction, variants were noted although other biochemical characteristics were relatively constant. All strains studied were identified as type 'A' strain on the basis of colonial, morphological, biochemical and growth characteristics and sensitivity to penicillin. When grouped serologically, 38 strains were type- 1; 3 type-2; 1 type-11 and one was not determined serologically. Of the isolates from the cases of shipping fever thirtyfour strains were type-1 and 3 were type-2.

Thomson et al (1969) studied pneumonic pasteurellosis of cattle. Bacterial and viral nasal flora and levels of bactrial and viral antibody in serum and nasal sceretion of cattle with normal and abnormal body temperature and plasma fibrinogen were compared. The most significant feature of the bacterial flora was the higher frequency of Pasteurella.haemolytica, specially when isolated two weeks after shipment.

Magwood et al (1969) studied nasal bacterial flora of calves in healthy but pneumonic pron herds. He classified the microorganisms into basal, supplementary and transient groups. Pasteurella multocida and Pasteurella haemolytica along with

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other organisms composed the basal flora and were widely distributed in cattle population. Enzootic pneumonia of calves was not associated with any characteristic bacterial flora but in cases of bacterial pneumonia in a well managed herd, Pasteurella. haemolytica was isolated from young animals.

Wang et al, (1969) studied an outbreak of haemorrhagic septicaemia of cattle in Georgia and Ethiopia. Pasteurella.multocida strain belonging to Cartter's capsul group 'E' and Namioka groups were isolated.

Nangyalai (1969) isolated 4.9% of Pasteurella multocida alongwith other microorganisms from the respiratory tract of 82 calves.

Gourlay et al (1970) examined lungs of 45 apparently healthy calves which were aged less than three months. He also examined 20 calves of different ages that had died or had been killed during extremes. He isolated 31.0% of Pasteurella species.

Hamnel (1970) isolated 28 strains of Pasteurella multocida of carter type 'E'. Potency test in mice revealed that they were more closely related to the African Carter Type 'B' than the European Carter type 'B' strain.

The Genus Staphylococcus.

Kumar (1965) recovered 41.5% of Staphylococci(Staph)along with other microorganisms from the nose of apparently healthy calves.

Elliot (1966) recorded the incidence of Staph., in cows in one herd and noted 14.0% Staph. aureus in the vagina, 5.5% in the rectum and 4.8% in nose.

Silberg (1966) studied the relative occurance of Staphylococcal infection in human beings and animals. No significant

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No significant difference in the occurrence of nasal infection owing to coagulase positive Staph. could be brought about between the faculty members exposed and not exposed to veterinary hospital and clinics (the overall incidence of nasal infection among out-patients was 20.0% as against 34.0% in hospitalised animals)

Pulverer and Entel (1967) recorded the incidence of coagulase positive Staphylococci in the nasopharynx of domestic and wild animals. Out of 608 domestic animals, 49.0% had nasaphyrynx infection (77.0% of horses, 57.0% of cattle, 53.0% of pigs, 47.0% of sheep, 46.0% of dogs and 15.5% of fowls). And out of 127 wild animals (deer, hares, rabbits, peasants and ducks) 23.0% had nasophyrynx infection. Horses and cattle had the highest countof Staphylococci.

Daleel and Frost (1967) studied 400 pairs of bovine tonsils and isolated 7.0% of coagulase positive Staph. alongwith other microorganisms. Singh and Mallik (1968) isolated Staphylococci from nasal cavities, trachea and lungs of buffaloes. The frequency of incidence of Staph. species isolated was 11%. He divided them further as follows Staph. aureus 3.1%, Staph. albus-5.2% and Staph. citreus 2.7%.

Nangyatai loc. cit. (1969) isolated 48.8% of Staph. along with other micro-organisms from the respiratory tract of 82 calves. Hajek and Marsalek (1970) isolated Staph. from the upper respiratory tract of different animal species and studied the physiological properties of Staph. aureus of porcin origin.

Schobesch et al,(1970) studied the value of colonisation of the nasopharynx of new born calves with pathogenic bacteria and their roles in the prophylaxis of digestive and respiratory

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diseases. The microflora present in the naso-pharynx of 1-6 day old healthy calves were coagulase negative Staph. alongwith other microorganisms (M.O.) Cultures of the microflora isolated from the above calves were introduced in the naso pharynx of 54 newly born calves. Enteric disorder subsequently developed in nine and respiratory infection in two of these calves. This result was compared with thirty normal newly born calves. E First of these had respiratory troubles where as all of them had digestive infection.

The Genus Streptococcus.

Davis (1923) discovered 24.0% of haemolytic Streptococci (Strept.) from 7 pairs of tonsils.

Kove and Waldman (1935) isolated Strept. from bovine infectious bronchitis. Ubertini (1939) isolated Streptococcus. pneumonia from the lungs of dead cattle.

Francis (1941) in a study of 100 pairs of tonsils from condemned heads of cattle, found coliform and Streptococcus to be the predominant organisms. He isolated Streptococcus.dysaglactiae 5.0%, Streptococcous uberiss-7.0% , haemolytic Streptococcus, group 'C'-9.0%, Streptococcus. salivaris 1.0% and enterococci 6.0%.

Glaser et al.,(1951) established pneumonia in white rats by instillation of beta haemolytic. Streptococci, intransally.

Geidel (1932) isolated 14.0% Streptococcus. pyogenes along with other microorganisms from the tonsils of 72 slaughtered cattle. Hammer (1953) isolated Streptococci. belonging to group D, E and L from calves having pneumonia.

Romar (1960) suggested Streptococcus. pneumonia to be the causative agent of infection in calves and was of the

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opinion that it is of great public health importance. Isolation of identical strains of the microorganisms from the lungs of the dying cows as well as the throats of the human attendants evidenced inter species transmission.

Obiger (1962) isolated a variety of bacteria from 124 pairs of bovine tonsils including Streptococcus. agalactiae 2.9%, Streptococcus. dysagalactiae 13.0%, Streptococcus. uberis 17.0% and Streptococcus. pyogenes 10.5%.

Payne and Derbyshire (1963) examined tonsils of 30 cows and 30 calves. They isolated a variety of bacteria including Streptococci but they did not fully differentiate them into serological groups.

Kumar (1965) isolated Streptococci 51.0% along with other microorganisms from the nasal cavities of healthy calves.

Daleel and Frost (1967) examined 400 pairs of cattle tonsils and isolated Streptococcus.dysglactiae 7.3%, Streptococcus. bovis 0.5%, Streptococcus.acidominis 1.25% and 16.7% of Streptococcus were no identified. Streptococcus. agalactiae was not isolated.

Singh and Mallik (1968) isolated Streptococcus.pyogenes 7.8% and other Streptococci 1.6% along with other organisms from the respiratory tract of baffaloes.

Magwood et al.,(1969) made an extensive study on nasal bacterial flora of calves in healthy and in pneumonic prone herds and classified them into basal, supplementary and transient groups. The potential pathogens Pasteurella. multicida and pasteurella. haemolytica and Streptococci along with other organisms composed the basal flora.

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Schobesch et al., (1970) isolated group D Streptococci along with other microorganisms from the nasal pharynx of healthy calves which were 1-6 day old.

The Genus Escherichia.

Pilot, Buck and Davis (1936) isolated Escherichia. coli (E.coli) associated with other organisms from 133 pairs of bovine tonsils.

Francis (1941) concluded that coli forms were the predominant organisms when he examined 100 pairs of cattle tonsils.

Leib (1951) isolated 92.0% of E.coli from 121 pairs of tonsils of apparently healthy calves and 27 pairs of sheep.

Kumar (1965) isolated 22.5% of E. coli along with other microorganisms which constituted 77.5% of his isolates from nares of 8 healthy calves.

Belchev et al.,(1967) isolated pure cultures of E. coli from the nasal mucosa of 80.0% of the calves. In this case 353 isolates were obtained from calves that had died between 1 and 10 days of ages on 20 farms.

Singh and Mallik (1968) isolated 8.9% of E. coli along with other microorganisms from the respiratory tract of baffaloes slaughtered at different abbatoirs.

Nangyalai (1969) isolated 15.8% of E. coli along with other microorganisms from the respiratory tracts of calves.

Gourlay et al., (1970), isolated E.coli from lungs of apparently healthy calves of different age groups which had died or or had been killed in extremes.

Ewing et al.,(1963) typed 320 strains out of which 85.0% were isolated from human beings and 15.0% from animals. Serotypes

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055, K 59 (B5) and H19 were isolated from only one human being.

Lovell (1955), Gay (1965), Sojka (1965) and Kolyakov etal., (1970) suggested that certain serological types of E.coli were associated with colibacteriosis in calves but the majority of these studies had been confirmed to strains isolated from the septicaemic form of the disease. Serotypes 078 and K80 had been most frequently found among strains isolated from internal organs of calves that died of coli infection. Representative of other "O" groups more frequently encountered, included 15,26,35,86, 115, 117, and 137.

The Genus Salmonella.

The causative agent of enteric fever was recovered by Eberth in 1880 and it was latter named as Eberthella typhosum by Gaffky in 1884. For the 1st time the organism was isolated in pure culture.

Gaertner (1888) in Germany isolated S.enteritidis(then called Bacillus enteritidis) from a human being who ate beef and died. By the end of the Nineteenth century two French workers (Archard and Besande, 1896) isolated Salmonella. paratyphoid from sample of urine, collected from patients with clinical symptoms of typhoid fever. This organism was latter named after Schottmuller who studied it in detail in 1900.

Bosworth and Lovel (1931) reported two outbreaks of Salmonella. dublin (S.dublin) infection in calves. Predominent clinical symptoms were diarrhoea and pneumonia.

Shirlaw (1935) isolated Salmonella from septicaemic diseases of calves and cattegorised them to be S. enteritidis which was latter confirmed by Raj Gopalan 1938 as S.dublin

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Leib (1951) investigated 121 pairs of tonsils from apparently healthy calves and 27 sick calves, paying special attention to the isolation of Salmonella. He failed to isolate any Salmonella from these animals.

Geidel (1952) also failed to isolate Salmonella from the tonsils of 72 slaughtered cattle.

During the period from 1958- 1960, Gibson in Britain listed the isolates of the Genus Salmonella collected from calf materials at veterinary institutes/ centres in England and Wales and confirmed that S. dublin was the predominent serotype of 918 strains isolated during this period, 573 (62.0%) were S. dublin and 313 (34.0%) S. Typhimurium. The other 32 strains consisted of 12 different serotypes.

Anderson et al., (1961) found S. typhimurium to be the predominant serotype when he examined the British calves at slaughter houses, and found that young calves were more susceptible to salmonellosis than adults. S. dublin and S. typhimurium caused the majority of outbreaks.

Thomas (1961) in Belgium found that S. dublin and S. typhimurium were equally prevalent in cattle.

Zagaevs Kii (1962) found that 70 (56.0%) of 124 strains isolated from cattle were S. dublin, 24 (19.0%) were S.typhimurium and 8 (6.3%) S. enteritidis.

Rude (1963) isolated 46 strains of Salmonella in cattle caused by S. typhimurium.

Prasad (1965) isolated S. weltereveden in pure culture from a pulmonary abscess in calf. The calf was suffering from skin disease. There was no symptoms of gasteroenteritis.

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Daleel et al., (1967) isolated S.muenchen from tonsils of three beef cows and one steer.

Mallick et al., (1959-1968) isolated S. typhimurium 1.44% and S. enteritidis 0.95% Salmonella species 0.24% from 980 sheep showing pneumonic lesions.

Gourlay et al., (1970) isolated two strains of S.epidemidis and 1 strain of S.bovis from 45 apparently healthy veal calves showing microscopic areas of pneumonia.

The Genus Fungus.

Ainsworth and austwick(1959) discussed pulmonary aspergillosis in Bovine. Eggert and Romberg (1960) reported the same.

Narain et al., (1964) isolated Aspergillus from a murrah she-buffalo. Cordes et al (1964) reported 13 cases of acute mycotic pneumonia in bovine.

Sudaric (1965) reported pneumonia in horses caused by Aspergillus which was identified as the causative agent in two horses which had post-mortem lesions of glanders. The condition was diagnosed histologically and acute tissue lesions and mycellium were present in the lungs. An unspecified Aspergillus was isolated from one of the horses. Severe oedema in the pulmonary parenchyma was positively of an allergic nature.

Munday (1967) reported mycotic infection in cattle and observed that mycotic infections are the most frequently diagnosed cause of abortion in Tasmania. Mortierella species and unidentified fungi have caused acute pneumonia and Aspergillus species chronic pneumonia in cattle.

Harcourt & Thomson (1969) reported mycotic abortion and

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mycotic pneumonia in a cow; two days after aborting during the eighth month of pregnancy. The cow had symptoms of metritis and the respiratory sounds were normal. Antibiotic treatment failed and so the cow was killed. On P.M. Examination metritis was observed and consolidation of anterior half of both lungs with many small whitis yellow granulomatous lesions through out the affected areas were seen. A fungus was isolated both from samples of the lung and from the stomach contents of foetus where hyphae were visible under microscope. Hyphae were clearly seen in sections of lung only when staining with cilestin blue was done. The fungus appeared to be Mortierella.

Augus and Gilmour (1970) emphasised on the occurrence of mycotic lesions in calves experimentally dosed with Mycobacterium avium (M. avium). Histopathological studies were carried out on the portion of small intestine and mesentric lymph nodes of animals which had been orally infected with M. avium. Ten or thirteen months after one strain of Avian tuberculosis was detected although mycotic granulomas associated with Aspergillus. fumigatus (A.fumigatus) and several other granulomatous lesions were seen.

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The Genus Mycobacteria.

Taneza (1955) reported incidence of T.B. in cattle of 1-3 years of age upto 8.0% and upto 45.0% in 4 years and older.

Saroop (1952) suggested a high incidence of tuberculosis in female calves than in male calves.

Viallier et al., (1966) emphasised the characteristic of M. bovis. Biochemical and biological studies done on the strains collected from 10 months to over 10 years, indicated that M. bovis retained its identifying characteristics.

Dnorvedi & Singh (1966) discussed the gross and microscopic lesions of tuberculosis in 12 cases of buffaloes and indicated its implication regarding the Veterinary and Public Health.

Tuboly and Szalio (1967) isolated M.tuberculosis from cattle which reacted positively to mammalian tuberculin in a T.B. free farm herd. From a small necrotic focus in lymph node, an acid fast organism was isolated and identified.

Lesslie et al ., (1966),(1970), isolated M.avium and M.bovis from tuberculoid cattle. Specimen from 14439 cattle, which were reactors to the single intra-dermal comparative T.B. test, were examined for T.B. for 16 years from 1953 to 1968. From 371 of these animals M. avium was isolated, representing 12.7% of the total of 2917 strains of T.B. bacilli isolated. Except for 13 isolates of M. tuberculosis the remaining infection comprising 87.0% of the total were all of bovine type.

Rathore et al., (1969) studied on the incidence and pathology of pulmonary T.B. in buffaloes. Pulmonary T.B. was observed in only 4(0.19%) of 1108 lungs of buffaloes slaughtered at Bareilly and Rampur abbatoirs (U.P.). Macroscopically, the

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lungs showed discrete, coalescing or capsulated tubercles measuring upto 2 c.m. in diameter. Microscopically, the lesions showed central areas of caseous calcification, surrounded by epitheloid cells, macrophages, giant cells of Langerham, lymphocyts and monocyts. Tubercle bacilli were demonstrated in the cells by the Z.N. staining procedure.

Lall et al., (1969) showed incidence of T.B. in cattle in India. The percentage of positive reactors to the tuberculin test was 1.93 among cattle and 6.39 among buffaloes.

Katikaridis (1969) made a survey of T.B. among slaughter animals from 1961 to 1965 in German Fedral Republic and from 1961 to 1967 in Munich. In 1968, T.B. was positive in 0.25% of 62,884 slaughtered cattle at Munich abbatoir. Lymph nodes affected were examined and ginea pigs (G.pigs) were inoculated. Mycobacteria were isolated from 106 cattle that is from 67.8% of the reactors. Among them M.bovis was recorded from 22 cases; M. avium from 80, mixed infection of M.bovis and M.avium from two and saprophytic ones from two cases.

Zorawkin (1969) studied biological, cytochemical and biochemical properties of Mycobacteria isolated from cattle. Biological tests in G.pig, rabbits and chickens together with neutral red, nile blue and niacin tests were carried to type 56 isolates from cattle and 1 from cow boy. Nine strains typed were M. avium, 1 strain isolated from the cow boy was typed as M. tuberculosis; others were all M.bovis.

Other Microorganisms.

Schimid (1933) attributed Corynebacterium. pyogenes (C.pyogenes) as a causative agent of calf pneumonia which occured in the form of a serious outbreak.

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Flatla (1942) discovered C. equi as the causative agent of pneumonia and cough in foals in age group of 1-4 months.

Bosworth and Lovell (1944) recovered haemolytic coccobacilli from the nasal cavity of goats, cattle and sheep suffering from nasal catarrah.

Heltman (1945) recovered C. equi in pure culture from purulent lesions of the lungs of a young calf which died of chronic pneumonia.

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

Geidal isolated 11.0% C.pyogenes from tonsils of 72 slaughtered cattle.

Payne and Derbyshire isolated C.pyogenes from the nose of cattle.

Singh and Pernaik (1965) isolated Corynebact (11.8%), Pseudomonas (8.5%), and Klebsiella species (6.5%) from the upper respiratory tract of calves.

Puschur and Toepfer (1967) studied on the occurrence of Pseudomonas. aeruginosa in the slaughtered animals. In routine and bacteriological meat inspection of animals with various diseases at Munich abbatoir over an eighteen months period, Pseudomonas. aeruginosa was isolated from 63 calves, 26 pigs and 1 sheep. The commonest lesion in infected calves (40.0%) was pneumonia. The commonest lesion in infected pigs were pneumonia (43.0%), and abscesses (19.0%). The sheep had also pneumonia.

Singh and Mallick (1968) isolated Citrobacter (2.7%), Klebsiella (0.5%), Proteus (2.5%), Alcaligenes (6.0%) and

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Corynebacterium (9.0%) along with other organisms from the respiratory tract of buffaloes.

Storz (1968) isolated Psittocosis. lymphogranuloma venerium agent from bovines suffering from pneumonia.

Wilson and Thomson (1968) isolated a Chlamydia agent from the lungs of a calf with enzootic pneumonia.

Sudakove (1968) did typing of Klebsiella strains isolated from animals. 305 Klebsiella strains isolated from mucous membrane of upper respiratory tracts of 2208 pigs and 564 cattle were studied. Only 18 of 34 bovine strains could be typed. These were all from healthy cattle and belong to types k-2, K-3, K-8 and K-9.

Robert (1969) isolated C. haemolyticum from a case of ovine pneumonia.

Magwood et al., (1969) studied nasal bacterial flora of calves in healthy and in pneumonic prone herds. Their nasal bacterial flora was classified into basal, supplementary and transient components. Haemophillus. influenza, Diplococcus pneumoniae and C. pyogenes together with other organisms composed the supplementary components whereas E. coli, Klebsiella pneumoniae formed the transient components.

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MATERIALS AND METHODS

Animals from which microorganisms isolated were mostly selected from slaughter house; the Surgery section of Bihar Veterinary College, Patna; Livestock Research Station (L.R.S.), Bihar, Patna and autopsy cases brought for P.M. examination in the College as given in Table I and Table-II.

TABLE I

Sources of Animals for Isolation of Microorganism.

Serial No.	Sources	Species of animals				
		Cows	Bullocks	Calves	Buffaloes	Buffalo calves
1.	Slaughter house Sahganj, Patna.	26	36	5	23	0
2.	Surgery Section, B.V. College, Patna.	0	0	0	0	36
3.	L.R.S., Patna	0	0	0	0	14
4.	Autopsy cases.	2	1	30	0	0
	Total	28	37	35	23	50

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TABLE II

Sources and Types of Samples from Which Microorganisms were isolated.

Serial No.	Sources	Region of the respiratory tract of animals from which cultures were done.			
		Nasal cavity	Trachea	Lungs	Total
1.	Slaughter house Sahganj, Patna	90	119	175	384
2.	L.R.S. Bihar, Patna	16	0	0	16
3.	Surgery Section, B.V. College, Patna.	45	0	0	45
4.	Autopsy cases	12	47	47	106
	Total	163	166	222	551

A. Collection of materials from living animals:-

The external nares of cattle before slaughtering were sterilised by alcohal and the sterilised swab was passed through the nares to a point posterior to the ostium of the maxillary sinus. By rotating the swab while moving it back and forth it was possible to ample the inferior meatus over an area 8 to 10 cm long. A composite swab was taken representing the right and left meatus. The animal number was noted for identification.

The materials were enriched immediately by inoculating into the broth solution which was kept ready below the tube for this purpose. The material adhering on the swabs were thoroughly mixed with the media and these tubes were incubated aerobically at 37°C. for six to eight hours. After incubation, a loopful of

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the broth culture was inoculated on blood agar plate (BAP) and incubated aerobically at 37°C. for 24 to 48 hours. The growth on the media was examined for pigment production, colonial characters and for haemolysis. The noticeable colonies were picked up to study the morphology, biochemical and physiological and other characteristics.

B. Materials from slaughtered/dead animals:-

The nasal (from dead animals) and tracheal swabs were collected from these animals. The swabs from trachea were collected by introducing them aseptically into the upper part of the trachea and rubbing it well into the middle and lower part of trachea. The swabs collected were processed in the same manner as described in (A) above.

C. Collection of materials from lungs:

The inoculum was prepared according to the modified technique of Collier and Rossow (1964).

Portions of lungs from the animals used in 'A' and 'B' were collected in a manner so that one piece was taken from an apparently healthy area and an other from pneumonic areas of lungs. The lung specimens were singed with a hot sterile spatula over the outer surface of the organ, a fissure was made with a sterilised pair of scissors and culture was made on BAP and subsequently studied as stated earlier.

Identification of bacteria.

It was carried out according to the methods prescribed in the " Bergey's Manual of Determinative Bacteriology "(Breed et al., 1957) and as per the tests recommended in the " A Manual

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of Bacteriology" (Cruickshank 1965).

Staphylococcus:

Organisms from a tipical colony on BAP were examined for morphology by Gram's method. If found to be Staphylococcus their colonial characteristics were studied by growing them on nutrient agar plate.

1. Catalase Production: The M.O. were grown on agar plates overnight. A few drops of 3 per cent hydrogen peroxide were flooded on colonies. The production of gas bubbling was taken as positive for catalase enzyme.

2. Coagulase Test.

Citrated rabbit plasma was diluted one in ten with isotonic saline solution. An amount of 0.5 ml. of the diluted plasma was taken in a test tube and five drops of fresh broth culture was mixed with it. The test was read at an interval of 2, 4 and 6 hours for Coagulation of plasma. If negative, the culture was left overnight at room temperature. Formation of fibrin clot indicated positive reaction.

3. Haemolysis.

Microorganisms were grown aerobically on bovine BAP at 37°C. for 24 hours. Wide zone of clear haemolysis, a narrow zone of incomplete haemolysis and a narrow zone of clear haemolysis around the colonies were considered respectively as alpha, beta and delta haemolysis. Those colonies having no change on BAP were taken as nonhaemolytic.

4. Pigment Production.

Pigment production was noted on agar slants after 24-48 hours of incubation at 37°C. and then latter kept at room

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temperature for 24 hours to observe the pigmentation if any.

5. Liquefaction of Gelatin.

Liquefaction of gelatin was also observed, as described in the " A Manual of Bacteriology" (Cruickshank 1965).

The Genus Streptococci.

The methods for study of morphology, colonial characteristics, catalase and haemolysis were followed as far staph.

Fermentation reaction lactoac, trehalose, mannit, inulin, sorbitol and salicin were carried out. Hydrolysis of sodium hippurate was also observed.

The Genus Escherichia.

The lactose fermenting round, translucent and convex colonies of about 2 mm. diameter that developed on MacConkey's lactose bile agar (M.L.A) plates were picked up and streaked on the Eosine Methylene blue agar (EMB) plate and incubated at 37°C for 24 hours. Colonies of moderate size with dark (almost black) centres and showing metallic sheen in light were cultivated on the nutrient agar plate (NAP) for the study of the colonial characters. Other half of the same remaining colony as transferred into nutrient broth for testing morphology and motility.

The isolates were maintained on agar slants for study of biochemical properties, indole production, methylene reduction (MR), production of acetyl methyl carbinol (VP), reduction of nitrate to nitrite, citrate utilisation (koser 's medium), gelatin liquefaction and production of hydrogen sulphide. Sugar fermentation tests were carried out using dextrose, lactose, maltose, sucrose, raffinose, dulcitol, adonitol, inositol, mannit and salicin. Acid and gas formation were detected ~~in the~~

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Serological Typing.*

The strains after identification were examined for their 'O' antigen. 44 hyper immune antisera comprising six polyvalent 'O' groups were used for serological typing of E.coli as detailed below:-

P1 - o1, 02, 011, 020,022, 071, 078, 0117.

P2 - 04, 06, 08, 026,073, 0101, 0141,

P3 - 03, 05, 09, 015,018, 041, 088.

P4 - 045, 053, 055, 061, 067, 0131, 0137.

P5- 035, 054, 069, 083, 0121, 0131, 0147.

P6 - 087, 0115, 0119, 0126, 0127, 0128 and 0142.

The 'O' antigen was prepared as per the modified method described by Kauffman (1947).

For the preparation of 'O' antigen, the 18 hours growth on agar slant was washed with 0.5 to 1 ml physiological saline and then it was heated for one hour in boiling water. The tube agglutination test was done as per the methods described in the "Manual of Microbiological Methods" (1957). The tubes were incubated for 3 hours at 52°C in a water bath. The premilinary readings were recorded and kept in refrigarator at 4°C for over night. The final readings were noted in the next morning. A tube was taken as the highest which showed a 50% agglutination.

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* The author is thankful to Dr.S.Kumar,Head of Division and Dr.S.P.Singh, Assistant Professor, of the Indian Veterinary Research Institute(I.V.R.I.),Izatnagar/ Mukhteswar (U.P.) for giving time and help in doing the serological work there.

The Genus Salmonella.

For isolation of Salmonella, the techniques advocated by Edwards and Ewing (1962) were used. For enrichment, tetrathionate broth with brilliant green and bile was used. After enrishment, it was inoculated on brilliant green agar (BGA) and MacConkey's agar plates (MAP).

The following sugars were used for fermentation reaction: lactose, dextrose, sorbitol, sucrose, dulcitol, adonitol, inositol, mannitol and salicin. Other biochemical studies were done as already explained with reference to E.coli. Christensen urease medium as modified by Hormache and Munnilla (1957) was used for testing of urease activities.

Serological typing * was done by the standard method of agglutination as described in the " Manual of Microbiological Methods (1957) using genus specific 'O' and phase-1 flageller antisera.

The Genus Klebsiella.

The identification and differentiation of Klebsiella from Escherichia were based on

(a) Morphology,

(b) Cultural Characters,

(c) Motility and

(d) Biochemical tests, especially Methyle red (M.R.), Vogues. Prauskaur (V.P.) test, production of indole and citrate utilisation test.

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* The author is thankful to Dr.S.P.Singh, Assistant Professor, I.V.R.I. Mukhteswar- Kumaon (U.P.) for giving time and help in doing the serological work there.

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The Genus Pseudomonas.

The pale yellow coloured colonies on BGA and MAP and also on blood agar were taken from the plates. These colonies were subcultured on plain agar slants.

The tubes showing green pigments changing to brownish after sometime was processed for the identification of Pseudomonas. To study haemolysin activity, the microorganisms were inoculated on BAP. The motility was checked by the hanging drop method. Indole production, M.R., V.P., Nitrate reduction, H_2S production, citrate utilisation test, catalase test and metheylene blue reduction test were carried out in the same manner as for other bacteria. Sugars used were dextrose, lactose, sucrose, maltose, arabinose and mannitol.

The pigment production was checked by treating the nutrient agar slant with chloroform. The greenish blue colour obtained was activated with hydrochloric acid to achieve the red coloration indicating the presence of pigment pyociamine which is the characteristic of microorganisms of this group.

The proteolytic activity of the isolates was checked by growing them on serum agar plate and by observing the digestion of the serum incorporated in the medium.

The Genus Proteus.

Discrete colonies on BAP with characteristic spreading tendency were subcultured on nutrient agar slants. Their morphological, cultural and biochemical characteristics were studied as for other organisms.

The Genus Corynebacterium.

For isolation of Corynebacteria serum agar was used. For

For morphological study, the culture smear was stained by the Albert's method as modified by Laybourn (1924).

Cultural characters were studied on the potassium tellurite blood agar medium whereas pigment production was studied on nutrient agar slants. Motility and biochemical tests were carried out as for other organisms.

The Genus Pasteurella.

Morphological study was carried out on young, eighteen hours broth culture which was incubated at 37°C. The smears were separately stained with Gram's and Giemsa's stain and then examined. Motility was observed in haging drop preparation from the broth culture. For cultural characters, the isolates were cultivated on NAP.

Biochemical tests were done as for the other microorganisms. Methylene blue reduction test was also performed. The pathogenocity trials for the isolates were done by inoculating 18 hours growth intraperitoneally (i/p) and subcuteneously (s/c) in rabbits and s/c in mice.

The Genus Mycobacteria.

From the portion of lungs showing caseation or abscesses smears were prepared and stained by the Ziehl Neelsen (Z.N.) method. If the tissue impression smears were positive for acid fast organisms, the lungs tissue was processed for isolation and identification of Mycobacteria. The lungs tissue was processed for isolation according to modified Petroffs method.

The portion of the lung was triturated in a sterilised pestle and mortal containing sterilised sand. In course of trituration equal volume of 4.0% of sodium hydroxide was thoroughly mixed by

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pouring drop by drop. The contents of the pestle and mortar was poured before the flame into 2 MacCartney's sterilised bottles and was then placed in incubator at 37°C for half an hour. The mixture was then centrifused at 3000 rpm. for 30 minutes and the supernatent was poured off. The deposite was neutralized with 8.0% hydrochloric acid, which was added drop by drop, the reaction of the mixture being tested by adding a drop of phenol red solution to the tube. The deposite was examined microscopically and was then inoculated on Lowenstein- Jensen medium slope (modified by Jensen, 1955 by omiting starch and inoculating malachite green). For each sample two culture tubes were incubated at 37°C for 3-5 weeks.

The organisms were grown in 5.0% glycerin broth medium at 37°C for four weeks.

Biochemical test were carried out as follows:-

i. Niacin test.

Carried out according to Marks and Trollope (1960).

ii. Neutral Red Test.

According to Dubos and Middle brook (1948), the test was performed.

iii. Catalase test.

The catalase activity was also observed as detailed before.

iv. Pigment production.

It was detected on coagulated serum.

Pathogenicity test.

G. pigs and rabbits were inoculated I/p and S/c with each culture obtained and mice were inoculated I/p.

(a) G.pigs. 0.1 mg of the culture was inoculated to each

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of the two G.pigs. One was inoculated S/c in the left thigh and the other was inoculated I/p and kept in isolation.

(b) Rabbits.

1.0 mg of the culture was inoculated S/c into the thigh of the rabbit. After death of the inoculated animals smears from organs were stained by the Z.N.method and processed again as before for isolation and cultivation of the organism. After 2-3 months if the animals survived, were killed and organisms were isolated and identified as before.

(c) Mice.

1.0 mg of the culture injected I/p into the mouse.

(d) Chichens.

Only 4 chickens were inoculated I/p.*

The Genus Aspergillus.

The isolates were examined unstained as well as lightly stained by the Gram's method. The mycelium was demonstrated in films of exudate mounted in 10-20 % sodium hydroxide.

For cultural character, Sabouraud's glucose agar media with and without chloromycetin were used. Each suspected sample was inoculated in the above two media for study.

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* The entire bacteriological work on Mycobacteria was done at the T.B.Demonstration & Training Centre,Patna under the guidance of Dr.K.K.Sinha, Director of the Centre.

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RESULTS

In the present study 173 heads of cattle and buffaloes, both living and dead, were taken up for isolation of microorganisms from their respiratory tracts. The results are recorded in Table III.

Nasal swabs before slaughter were taken from living animals and nasal, tracheal and lungs swabs were collected from the dead and slaughtered animals for isolation of microorganisms(M.O.). From fifty living buffalo calves, only nasal swabs were taken for isolation of m.o.

A total of 520 isolates of different genera of m.o. were collected as presented in Table IV and figure 1.

The genuswise description of each isolate is presented below:-

The Genus Escherichia.

After 24 hours of incubation at 37°C on agar plates, the morphological appearance of colonies were circular, lowconvex, smooth and colourless. In broth culture the growth was abundant with an uniform turbidity. On MacConkey's lactose agar plates, smooth convex and pink colonies were found. On Eosin methylene blue (EMB) agar plate, the colonies were of moderate size with dark (nearly black) centres and the confluent growth showed mettallic sheen against reflected light.

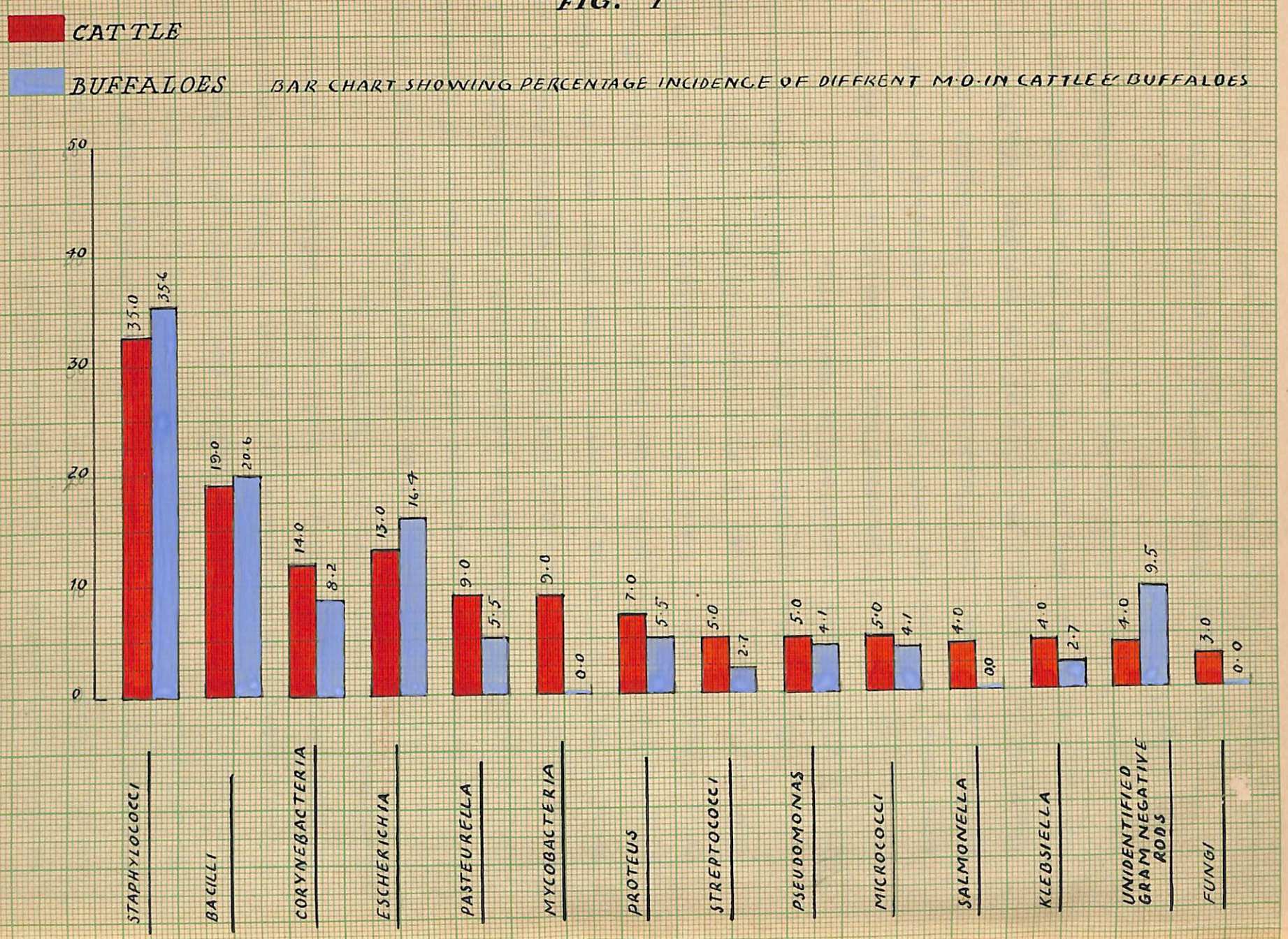
Microorgnisms were Gram negative and 0.8 to 1.2 microns x 2.0 to 4.0 microns in size.

TABLE III

Frequency of Isolation of Microorganisms from Respiratory Tracts of Cattle and Buffaloes

Source	Gm. -ve rods								Gm.+ve rods			Gm.+ve cocci				Other m.o.			Percentage
	Pasteurella	Salmonella	Klebsiella	Escherichia	Pseudomonas	Proteus	Unidentified Gm.-ve rods	Total	Corynebacterium	Bacillus	Total	Staphylococcus	Streptococcus	Micrococcus	Total	Mycobacterium	Fungi	Total	
Nose	7	-	2	32	11	6	8	66	5	32	37	76	3	11	19	-	-	193	37.5
Trachea	12	1	2	18	9	13	6	61	11	38	49	34	3	9	46	4	2	162	31.2
Normal lungs	2	-	-	3	1	-	2	8	-	12	12	12	-	1	13	-	-	33	6.30
Pneumonic lungs	12	4	4	11	8	1	11	51	18	8	26	27	7	9	43	9	3	132	25.0
Total	33	5	8	64	29	20	27	186	34	90	124	149	13	30	192	13	5	520	100.0
Percentage of total No. of isolates	6.34	0.96	1.5	12.3	5.6	3.8	5.2	35.7	6.5	17.5	23.8	28.7	2.5	5.8	37.0	2.5	0.96	100.0	100.0

FIG. 1


CATTLE
BUFFALOES
BAR CHART SHOWING PERCENTAGE INCIDENCE OF DIFFRENT M.O. IN CATTLE & BUFFALOES
50
40
30
20
10
0
35.0
35.6
19.0
20.6
14.0
8.2
13.0
16.4
9.0
5.5
9.0
0.0
7.0
5.5
5.0
2.7
5.0
4.1
5.0
4.1
4.0
0.0
4.0
2.7
4.0
9.5
3.0
0.0
STAPHYLOCOCCI
BACILLI
CORYNEBACTERIA
ESCHERICHIA
PASTEURELLA
MYCOBACTERIA
PROTEUS
STREPTOCOCCI
PSEUDOMONAS
MICROCOCCI
SALMONELLA
KLEBSIELLA
UNIDENTIFIED GRAM NEGATIVE RODS
FUNGI

Bichemical reactions were presented in Table V. Indole was produced, methyl red reactions was positive and acetyl methyl carbinol (V.P.) reaction was negative. Citrate was not utilised except isolates No.11, H_2S was produced only by strain 10 and gelatin was not liquefied. Biochemical patterns of E.coli isolated from cattle and buffaloes are summarised in table VI. Classification of the patterns(strains) is based on fermentation of different sugars and the physiological activities of these isolates as already presented in Table V.

Six isolates out of 21 isolated from living buff calves belonged to pattern no.3 while six isolates out of 19 isolated from dead calves belonged to pattern no.1. It is interesting that 37 isolates from apparently healthy and slaughtered animals were grouped in 11 different patterns. On the other hand 10 different biochemical patterns were found in 27 isolates recovered from dead animals. Thus a total of 11 biochemical patterns or strains were established because 10 were common between living and dead.

Serological grouping of E. coli isolated from dead and living animals was summarised in Table VII.

The table illustrated that 48 isolates which could be serotyped (16 isolates could not be serologically matched with existing antisera) are distributed in 14 serological groups based on "O" antigens. Majority of the isolates (57.1%) fall in serological groups 086, 0119, 0126, 09, 026, and 055 in order of frequency. Serotype 086 (20.9%) was the largest group followed 0119 (16.2%).

Fortyeight isolates of E.coli which fell in 14 serological groups and 11 biochemical patterns were further examined for

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TABLE V

Biochemical properties of E. coli Isolated from Cattle and Buffaloes

Group	Dextrose	Lactose	Maltose	Sucrose	Raffinose	Dulcite	Adonite	Inosite	Mannite	Salicin	Indole	* Hydrogen sulphide	Gelatinase activity.	Koser	Vogesproskauer	Methyl red	Urease activity
1	AG	AG	AG	-	-	-	-	-	AG	-	+	-	-	-	-	+	-
2	AG	AG	AG	A	A	A	-	-	AG	A	+	-	-	-	-	+	-
3	AG	A	A	A	-	A	--	-	A	-	+	-	-	-	-	+	-
4	A	A	AG	-	A	-	-	-	AG	A	+	-	-	-	-	+	-
5	A	AG	AG	A	A	A	-	-	A	A	+	-	-	-	-	+	-
6	AG	A	AG	-	-	-	-	-	AG	-	+	-	-	+	-	+	-
7	A	A	AG	-	A	A	-	-	AG	A	+	-	-	-	-	+	-
8	AG	A	A	A	A	-	-	-	A	-	+	-	-	-	-	+	-
9	A	AG	AG	-	-	-	-	-	AG	A	+:	-	-	-	-	+	-
10	A	AG	AG	A	A	-	-	-	A	-	+	±	-	-	-	+	-
11	AG	AG	A	-	-	A	-	-	AG	A	+	-	-	+	-	+	-

* No. of isolates which produced H_2S faintly.

AG denotes acid and gas, A= acid, + = positive, - = negative, ± = weekly positive.

TABLE VI

Biochemical Pattern of E.coli Isolated from Cattle and Buffaloes

Source	No. of animals examined	Biochemical Patterns											Total Isolates
		1	2	3	4	5	6	7	8	9	10	11	
* Apparently healthy animals													
Bullock	1	1	-	-	-	-	1	-	-	-	-	-	2
Buffaloes	2	-	1	-	2	-	2	-	-	-	-	-	5
Buff Calves (Live)	12	1	2	6	1	1	2	2	2	1	3	-	21
Calves	2	1	-	1	1	1	-	1	2	-	-	2	9
Total	17	3	3	7	4	2	5	3	4	1	3	2	37
P.M.Cases													
Cow	1	2	1	-	-	1	-	-	-	-	-	1	5
Bullock	1	1	-	1	-	1	-	-	-	-	-	-	3
Calves	8	6	2	1	-	1	2	2	1	2	2	-	19
Total	10	9	3	2	-	3	2	2	1	2	2	1	27
Grand Total	27	12	6	9	4	5	7	5	5	3	5	3	64

* Apparently healthy animals were slautered in the public abbatoir.

TABLE VII

Serological Groupings of *E.coli* Isolated from Cattle and Buffaloes.

Source		Antisera														Total isolates typed	Total isolates untyped	Column Total
		08	09	015	026	035	041	055	078	086	0101	0115	0119	0126	0127			
Apparently healthy animals																		
Bullock	1	-	1	-	-	-	-	-	-	1	-	-	-	-	-	2	-	2
Calves	2	-	1	-	-	-	-	-	-	3	-	1	1	1	-	7	2	9
Buffaloes	2	1	-	-	-	-	-	-	-	1	-	-	1	-	-	3	2	5
Buffalo Calves	12	-	2	-	1	1	1	1	-	5	-	1	1	1	1	15	6	21
Total	17	1	4	-	1	1	1	1	-	10	-	2	3	2	1	27	10	37
Dead Animals																		
Cow	1	-	-	1	-	-	-	-	-	-	1	-	1	1	-	4	1	5
Bullock	1	-	-	-	-	-	-	-	-	1	-	-	-	1	-	2	1	3
Calves	8	-	-	-	3	-	1	2	1	-	-	-	5	2	1	15	4	19
Total	10	-	-	1	3	-	1	2	1	1	1	-	6	4	1	21	6	27
Grand Total	27	1	4	1	4	1	2	3	1	11	1	2	9	6	2	48	16	64

their comparative studies. The results are summarised in Table VIII.

TABLE VIII

Biochemical Patterns of Different Serotypes of E.coli Isolated from Cattle and Buffaloes

Source	Sero-types	Biochemical patterns 1	2	3	4	5	6	7	8	9	10	11	Total
Apparently healthy animals	08	-	-	-	-	-	-	-	-	1	-	-	1
	09	1	-	1	-	1	-	1	-	-	-	-	4
	026	-	-	-	1	-	-	-	-	-	-	-	1
	035	-	-	-	-	-	-	-	-	-	1	-	1
	041	-	1	-	-	-	-	-	-	-	-	-	1
	055	-	-	-	-	-	-	-	-	1	-	-	1
	078	-	-	-	-	-	1	-	-	-	-	-	1
	086	4	-	1	1	1	-	1	-	2	-	-	10
	0115	-	1	-	-	-	-	-	1	-	-	-	2
	0119	-	-	1	-	1	-	-	-	-	-	1	3
	0126	1	-	-	-	-	1	-	-	-	-	-	2
	Total	6	2	3	2	3	2	2	1	4	1	1	27
Dead animals	015	-	-	-	-	-	-	1	-	-	-	-	1
	026	-	-	-	2	-	-	-	-	-	1	-	3
	041	-	1	-	-	-	-	-	-	-	-	-	1
	055	-	-	1	-	1	-	-	-	-	-	-	2
	086	-	-	-	-	-	-	-	-	1	-	-	1
	0101	-	-	1	-	-	-	-	-	-	-	-	1
	0119	4	1	-	1	-	-	-	-	-	-	-	6
	0126	-	2	-	-	1	-	-	-	-	-	1	4
	0127	-	-	-	-	-	-	1	-	1	-	-	2
	Total	4	4	2	3	2	-	2	-	2	1	1	21
	Grand Total	10	6	5	5	5	2	4	1	6	2	2	48

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It was evident from Table VIII that 11 biochemical patterns (strains) are serologically grouped in 14 types. 086 accounted for the majority of strains recovered from apparently healthy animals followed by 0119 isolated from dead animals. The remaining serotypes were distributed between 1-4 strains.

The Genus Klebsiella.

A total of eight isolates belonging to the genus Klebsiella have been isolated from four cattle and two buffaloes. Out of 8 isolates, four were isolated from lungs and two isolates were from traches of cattle while two were from nostrils of buffalo calves.

All the eight isolated strains were Gram negative rods and non motile.

On agar plate, the colonies were raised, greyish white and mucoid in consistency. On MIA plates, the colonies were reddish and mucoid.

With regard to biochemical characters all the eight strains were M.R. negative; V.P. positive; indole negative and H_2S negative. All grew in Koser's citrate medium, and reduced nitrate to nitrites. All strains produced acid and gas in dextrose, lactose, maltose, sucrose, salicin, adonitol and inositol except dulcitol.

The Genus Salmonella.

Salmonella were isolated from the pneumonic lungs and trachea of two cattle brought for the post mortem (PM) examination at the B.V.College,Patna and from two bullocks brought for slaughter at the Patna Municipal Corporation, slaughter house, Patna. The incidence of Salmonella in cattle was 4.0% , while in buffaloes it was nil.

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Typical pink colonies from BGA plates or colourless colonies from MacConkey's plates were subcultured on NA plates for biochemical studies. After 18-24 hours growth on nutrient agar different media and sugars were inoculated respectively for biochemical tests and for sugar fermentation. The results were recorded in Table IX.

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TABLE IX

Biochemical Reactions of *Salmonella* Isolated from Cattle and Buffaloes

Sl. No.	Indole	Methyle red	Voges proskauer	Koser Citrate	Nitrate reduction	Hydrogen sulphide	Gelatinase	Urease activity	Dextrose	Lactose	Sucrose	Dulcite	Adonite	Inosite	Mannite	Salicin	Sorbitol
1	-	+	-	+	+	+	-	-	AG	-	-	AG	-	-	AG	-	A
2	-	+	-	+	+	+	-	-	AG	-	-	AG	-	-	A	-	A
3	-	+	-	+	+	+	-	-	A	-	-	A	-	-	A	-	A
4	-	+	-	+	+	+	-	-	AG	-	-	AG	-	-	AG	-	AG
5	-	+	-	+	+	+	-	-	AG	-	-	A	-	-	A	-	AG

Note: + positive; - negative; A denotes acid; AG denotes acid and gas.

Five strains of Salmonella were almost similar biochemically on the basis of fermentation reaction and physiological tests.

They were further tested with Salmonella group specific "O" antisera and phase-1 flagellar antisera. The results are presented in Table X.

TABLE X

* Serological typing of 5 strains of Salmonella Isolated from the Respiratory Tract of Cattle and Buffaloes.

Serial No.	Strain number	Antigenic structer	Species
1	171/S	9, 12: g,m	S.enteritidis
2	273/S	9, 12: g,m	S.enteritidis
3	285/S	9, 12: g,m	S.enteritidis
4	301/S	9, 12: g,m	S.enteritidis
5	309/S	9, 12: g,m	S.enteritidis

All these strains isolated from cattle were grouped as S.enteritidis on the basis of biochemical and serological reaction.

* The author is grateful to Dr.S.P.Singh,Assistant Professor, I.V.R.I.,Mukteswar Kumaon (U.P.) for serological grouping.

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The Genus Pasteurella

Thirtythree isolates belonging to the genus *pasteurella* were recovered from different organs of cattle and buffaloes as presented in Table XI.

TABLE XI

Occurrence of *pasteurella* in the Respiratory Tract of Cattle and Buffaloes.

Species of animals from which isolated	Nose	Trachea	Lungs		Total
			Normal	Pneumonic	
Apparently healthy					
Bullock	2	1	3	-	2	5
Cow	1	-	1	-	1	2
Buffaloes	3	2	3	1	3	9
Buffalo Calves	1	1	-	-	-	1
Total	7	4	6	1	6	17
Dead						
Calves	6	3	6	1	6	16
Grand Total	13	7	12	2	12	33

Out of 33 isolates of *pasteurella* recovered, 23 were from cattle and 10 were from buffaloes. *Pasteurella* was recovered from six (20.0%) dead calves out of 30 dead calves examined.

Morphologically they were small Gram negative straight rods or cocco bacilli and showed characteristic bipolar staining when stained with Giemsa's stain.

After 24 hours growth on agar plate at 37°C the colonies were round, low, convex, amorphous, transluscent and smooth with entire edge. On BAP there was abundant growth, haemolysis was absent, except in case of six isolates which were grouped as P.haemolytica.

Biochemical characteristics and sugar fermentation reaction are recorded in Table XII.

Twentyseven isolates of P.multocida fell into 4 biochemical patterns while six isolates of P.haemolytica broadly formed two strains.

Two (6.6%) dead calves yielded P.haemolytica and 4(13.3%) dead calves yielded P.multocida. The dead calves were brought for the P.M.examination at the B.V.College,Patna. Most of the isolates from dead calves were from pattern 3 but with regard to others there was no significant difference.

Pathogenicity test.

It was performed with only 3 isolates of P.multocida (Two isolated from dead calves and one from buffalo). An amount of 0.5 ml of overnight broth culture was inoculated i/p and s/c to a pair of rabbits. The former died at 20 hours and the latter at 30 hours after inoculation. After death of these rabbits, Pasteurella organisms with characteristic bipolar staining were isolated from heart blood, lungs, liver and spleen of these rabbits.

Pathogenicity test was also conducted in mice and pure culture of P.multocida was recovered from them after they succumbed to these injections.

When broth culture of P.haemolytica of 18 hours growth

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TABLE XII

Biochemical Properties of 33 Isolates of *Pasteurella* Recovered from Cattle and Buffaloes.

Group	Total No. of isolates	Indole	M.R.	V.P.	Citrate	Nitrates reduction	H2S	Litmus milk reduction	Gelatin liquefaction	Methylene blue reduction	Dextrose	Maltose	Sucrose	Dulcitol	Mannitol	Arabinose	Haemolysis	Species
1	4	-	-	-	-	+	-	-	-	+	A	A	A	A	A	-	+	*P.haemolytica*
2	2	-	-	-	-	+	-	+	-	+	A	A	A	-	A	A	+	*P.haemolytica*
3	12	+	-	-	-	+	+	-	-	+	A	-	A	-	A	A	-	*P.multocida*
4	8	+	-	-	-	+	+	-	-	+	A	-	A	-	A	-	-	*P.multocida*
5	4	+	-	-	-	+	+	-	-	+	A	A	A	-	A	A	-	*P.multocida*
6	3	+	-	-	-	+	+	-	-	+	A	A	A	-	A	-	-	*P.multocida*

was inoculated s/c into a pair of mice no death occurred.

The Genus Corynebacterium.

Thirtyfour isolates were assigned to the genus Corynebacterium according to their biochemical characteristics as presented in Table XIII. They were Gram positive, pleomorphic rods. All the isolates were non-motile, non-spore forming. Cells of Corynebacterium equi (C.equi) were comparatively thick.

In nutrient broth there was sediment in the bottom of culture tubes and cell colonies adhered to the side of culture tubes. After 24 hours of incubation on blood agar at 37°C the colonies were small, pin point, 1 mm in diameter. The growth on BAP was improved when potassium tellurite was added. On potossium tellurite blood agar plates small, black and sometimes greyish colonies were observed. They were recovered from different lesions as already cited in Table IV.

Out of 34 isolates of Corynebacterium, 27 isolates were C.pyogenes and seven isolates belonged to C.equi species. All the seven isolates of C.equi have been isolated from pneumonic lungs of dead calves. Thirteen cattle (13.0%) out of 100 examined yielded Corynebacteria whereas only six buffaloes (8.2%) out of 73 were positive for this group of organisms.

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TABLE XIII

Fermentation Reactions of 34 Isolates of Corynebacterium

Recovered from Cattle and Buffaloes.

Total No. of isolated	Growth on tellurite	Indole	Methyl red	Voges-proskauer	Hydrogen sulphide	Nitrate reduction	Citrate	Gelatin liquefaction	Catalase	Urease activity	Haemolysis	Dextrose	Lactose	Sucrose	Maltose	Mannite	Species
10	Black	-	-	-	-	-	-	+	-	-	+	A	(A)	A	A	-	C.pyogenes
7	Black	-	-	-	-	-	-	+	-	-	+	A	(A)	A	A	A	C.pyogenes
4	Black	-	-	-	-	-	-	+	-	-	+	A	(A)	-	A	-	C.pyogenes
6	Black	-	-	-	-	-	-	+	-	-	+	A	(A)	A	A	A	C.pyogenes
2	Slate	-	-	-	1+	+	-	-	+	+	-	-	-	-	-	-	C.equi
5	Gray	-	-	-	2+	+	-	-	+	+	-	-	-	-	-	-	

Note: 1 + denotes weekly positive; 2 + strongly positive

'A' acid produced after 48 hours.

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The Genus Staphylococcus.

The incidence of Staphylococcus (Staph) in cattle and buffaloes were presented in Table XIV and Table XV.

TABLE XIV

Incidence of Staphylococcus in Different parts of the Respiratory Tract of Cattle and Buffaloes.

Species of animals from which isolated	Organisms		Organs from which isolation was made: Nose	Trachea	Normal lung	Pneumonic lung	Total
Cattle	Staph. coagulase positive	No.	22	6	4	6	38
		%	56.5	15.5	10.5	15.5	40.8
	Staph. coagulase negative	No.	32	10	6	7	55
		%	58.1	18.1	10	12.7	59.2
Buffaloes	Staph. coagulase positive	No.	10	5	2	4	21
		%	47.6	23.8	9.9	19.0	37.5
	Staph. coagulase negative	No.	19	8	3	5	35
		%	54.2	22.8	8.5	14.2	62.5

TABLE XV

Classification According to Important Physiological Characteristics.

Species of animals	Pigment production		Coagulase activity		Haemolysin production		Gelatinase activity	
			No.	%	No.	%	No.	%
Cattle	Golden	25	22	88	22	88	21	84
	White	45	16	40.3	10	22.2	39	86.6
	Lemon Yellow	23	-	-	-	-	18	81.8
	Total	93	38	40.8	32	34.7	78	83.8
Buffaloes.	Golden	15	14	93.5	14	93.5	13	86.6
	White	29	7	24.1	8	27.5	21	72.4
	Lemon Yellow	12	-	-	-	-	9	75.0
	Total	56	21	38.5	22	93.0	43	76.7

Out of 149 isolates of Staphylococci from 100 cattle and 73 buffaloes, 93 were from 35 cattle and 56 were from 26 buffaloes. From cattle, 25 isolates were positive for golden pigment, 45 isolates were positive for white and 23 isolates were positive for lemon yellow pigment. From buffaloes, 15 isolates were positive for golden secretion, 29 for white pigments and 12 for lemon yellow. There were 38 isolates from cattle which were positive for coagulase whereas 21 isolates from buffaloes were positive for coagulase. Thirtytwo isolates from cattle and 22 isolates from buffaloes were haemolysin producing. 78 isolates from cattle and 43 isolates from buffaloes were positive for gelatinase

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activities.

Diplococci and Micrococci were also isolated from cattle and buffaloes as summarised in Table XVI.

TABLE XVI

Incidence of Gram positive Diplococci and Micrococci in the Respiratory Tract of Cattle and Buffaloes.

Organisms	Nose	Trachea	Lungs		Total
			Normal	Pneumonic	
Diplococci	5	4	1	4	14
Micrococci	7	5	-	4	16
Total	12	9	1	8	30

Out of 30 isolates, 18 were isolated from cattle and 12 from buffaloes.

The Genus Streptococci.

Biochemical characteristic of 13 strains of Streptococcus isolated from cattle and buffaloes were represented in Table XVII.

TABLE XVII

Biochemical Properties of Streptococcus Isolated from Cattle and Buffaloes.

No. of isolates	Lactose	Trehalose	Mannite	Inulin	Sorbit	Salicin	Sodium hippurate	Remarks
1	A	A	-	-	-	A	±	Lancefield group B
8	A	A	-	-	±	-	-	Subgroup c of group C of Lancefield
4	A	-	-	-	A	A	-	Subgroup b of group C of Lancefield

Note: ± denotes weekly positive.

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The colonies were discrete, small, circular and opaque in appearance. On BAP, betahaemolysis was commonly observed. When stained with Gram's stain, the cells were Gram positive and showed chain formation of the size of 5 to 10 cells.

Biochemical reactions of one isolate was similar to biochemical characteristics of cells of group B of Lancefield and 12 isolates were like those of group C. Out of these 12 isolates, eight belonged to subgroup c of group C of Lancefield and four isolates could be grouped in subgroup b of group C. Out of 13 isolates, five were isolated from pneumonic lungs of cattle and two from pnemonic lungs of buffaloes; two from cattle nose and one from nose of a buffalo whereas one from trachea of cattle and two from trachea of buffaloes. The summary was presented in Table XVIII.

TABLE XVIII

Occurrence of Streptococcus in Different Regions of the Respiratory Tract of Cattle and Buffaloes.

Provisional grouping of the isolates.	Sources of species	Nose	Trachea	Lungs	Pneumonic lungs.	Total no. of strains.
Lancefield group B	Cattle	-	-	-	1	1
Subgroup c of group C of Lancefield		1	1	-	3	5
Subgroup b of group C of Lancefield		1	-	-	1	2
Total		2	1	-	5	8
Subgroup c of group C of Lancefield	Buffaloes	1	1	-	1	3
Subgroup b of group C of Lancefield		-	1	-	1	2
Total		1	2	-	2	5

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The Genus Pseudomonas.

The incidence of Pseudomonas is recorded in Table XIX.

TABLE XIX

Occurrence of Pseudomonas in the Respiratory Tract of Cattle and Buffaloes.

Source	No. of isolates	Nose	Trachea	Lungs	
				normal	pneumonic
Cattle	17	6	5	1	5
Buffaloes	12	5	4	-	3
Total	29	11	9	1	8

Out of 520 isolates, 29 isolates were from the genus Pseudomonas of these, 17 isolates were recovered from cattle and 12 from buffaloes.

On nutrient agar plates after 24 hours of incubation at 37°C the colonies were large and spreading in nature. After 1-2 days of incubation the medium turned greenish yellow showing chromogenic form of cells. The cells were motile. In broth also yellowish green pigmentation was observed.

With regard to biochemical characters all the isolated strains were indole negative, M.R.negative, V.P.negative, H_2S negative, Nitrates not reduced to nitrites (most probably due to nitrogen was evaporated in gaseous stage); citrate utilised; catalase positive and methylene blue was reduced. Twenty isolates (68.9%) liquefied serum. Only 3 (10.3%) isolated from cattle fermented maltose and 18 isolates (62.0%), that is, 10 isolates from cattle and 8 from buffaloes, fermented dextrose but lactose, sucrose, mannitol and arabinose were not fermented.

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The Genus Proteus.

Out of 520 isolates, 20 strains of Proteus had been isolated from the repiratory tract of cattle and buffaloes as summarised in Table XX.

TABLE XX

Occurrence of Proteus in the Respiratory Tract of Cattle and Buffaloes.

Source	Nose	Trachea	Lungs		No. of isolates
			normal	pneumonic	
Cattle	4	7	-	1	12
Buffaloes	2	6	-	-	8
Total	6	13	-	1	20

The microorganisms were Gram negative rods, about 1 to 2.5 microns in length and 0.5 to 0.8 microns in width and were arranged singly or in pairs. Short chains and filamentous forms were also observed. On NAP, the growth was typical and characterised by swarming appearance and the odour was ammoniacal. Within 24 hours of incubation at 37°C, the growth was spread over the surface of the whole plate. In broth, turbidity was recorded and deposits were seen at the bottom of the tube after 24 hours of incubation at 37°C.

Biochemically all the 20 isolates were positive for production of indole, H_2S, gelatinase, urease and catalase and nitrates was reduced to nitrites. All isolates were M.R. positive and V.P. negative. All produced acid and gas in dextrose maltose, sucrose and salicin but lactose, dulcitol, adonitol inositol and mannitol were not fermented.

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The Genus Bacillus.

A total of ninety isolates of the genus Bacillus were recovered from the respiratory tract of cattle and buffaloes. Morphology of cells from culture media was as follows:-

Sporulated rods differing in size and with rounded ends. The percentage of incidence in different species is represented in Table XXI.

TABLE XXI

Incidence of Bacilli in the Respiratory Tract of Cattle and Buffaloes.

Source	Nose	Trachea	Lungs		Total No. of isolates
			Apparently healthy	Pneumonic	
Cattle	20	27	7	6	60
Buffaloes	12	11	5	2	30
Total	32	38	12	8	90

The Genus Mycobacterium *

A total of 13 isolates belonging to the genus Mycobacterium were recovered from the respiratory tract of cattle only. Two isolates were recovered from the lungs and trachea of a dead cow, brought for the post mortem examination at the B.V.College,Patna and 10 isolates were recovered from seven(3 males and 4 females) cattle out of 65 which were examined in the local slaughter house.

* The entire bacteriological work on Mycobacteria was done at the T.B.Demonstration & Training Centre,Patna under the guidance of Dr.K.K.Sinha, Director of the Centre.

Only one female calf (2.8%) out of 35 calves examined yielded T.B.bacilli. No Mycobacterium was isolated from the seventy three buffaloes examined for this study.

Morphologically, the organisms were Gram positive slender rods measuring 1-4 microns in length and 0.3-0.6 microns in breadth Some were arranged singly and some were arranged in small clumps. They were non motile and all were acid fast when stained with the Z.N. method.

The colonial characteristics and pathogenicity to laboratory animals are presented in Table XXII.

In glycerine broth medium after 4 weeks of incubation at 37°C, the growth was thin and slightly nodular. No luxurient growth was found. In Lowenstein Jensen medium (without glycerine) after 3.5 - 5 weeks of incubation at 37°C, the growth was dysgonic greyish white and friable.

Biochemically all the isolates were positive for catalase production, neutral red reaction except niacin test which was negative for all,% except one which was weekly positive).

Pathogenicity trials were conducted in G.pigs, rabbits, mice and chickens.

G.pigs.

After intraperitoneal injection to five G.pigs, they were observed until their deaths. One G.pig died on each of the 14th, 15th, 16th, 19th and 21st day of inoculation. The P.M. examination was conducted on their dead bodies, lesions recorded and smears examined for acid fast organisms and cultures were made in the L.J.medium.

When injected subcuteniously with the suspected materials

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TABLE XXII

Characteristics of Mycobacteria Isolated from the Respiratory Tract of Cattle

Source	Total No. of Isolates	Species of Mycobacterium	Colonial charactor Lowenstein Jensen medium	Pigmentation Growth in dark	Pigmentation Exposed to light	Growth rate	Neutral red	Niacin test	Catalase	Animal pathogenicity G.pig	Rabbit	Mouse	Chicken
Cows (Slaughtered)	6	M.bovis	Dysgonic, slightly moist, granular, friable	None	None	4-5 weeks	+	-	3 +	4 +	4 +	4 +	-
Cow(dead)	2	M.bovis	Do	None	None	3-5 weeks	+	-	3 +	4 +	4 +	4 +	-
Bullocks (Slaughtered)	4	M.bovis	Do	None	None	4.5-5 wks	+	-	1 +	4 +	4 +	4 +	-
Calf (Slaughtered)	1	M.bovis	Do	None	None	5 weeks	+	- +	3 +	4 +	4 +	4 +	-

Note:- Except the dead cow other animals from which materials were collected were slaughtered in the local slaughter house for beef for human consumption.

- denotes negative
- + denotes weekly positive
- 1 + denotes weekly positive
- 3 + denotes positive
- 4 + Strongly positive.

the G. pigs died after 5-7 weeks. Necrotic areas along with miliary tuberculosis in lungs were observed. The lymph nodes in course of drainage of lymph from the site of injection were always involved. The lesions are represented in figure 2 & 3.

Rabbits:

Six rabbits were inoculated. After s/c injection they died after a lapse of time from 2.0- 3.0 months. Generalised tuberculosis was observed involving lymph nodes, lungs, livers and kidneys. Tubercles in lungs were having caseation in some rabbits.

Mice:

Six mice were inoculated i/p with one milligram of culture. They died from 21-31 days post inoculation. On P.M. examination lungs were studded with gelatinous, miliary tubercles which were transluscent in nature.

Chickens:

None died after inoculation within three months of period.

The Genus Aspergillus.

Five strains of Aspergillus were isolated from lungs of 2 slaughtered cows and from one slaughtered bullock.

In Sabouraud's glucose agar slant after 4 days of incubation at 28°C, smoky green colonies were observed. In Sabouraud's agar with chloromycetin the same smoky green colonies appeared after 4 days of incubation at 28°C .

Details of the fungus were not studied.

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Fig.2. G.pigs showing necrotic areas and miliary tubercles in lungs.

Fig.3. Showing necrotic areas in lungs, liver and spleen of G.pigs.

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DISCUSSION

Studies on respiratory microflora in mammals have been few and fragmentary. In some instances, the carrier rates have been assessed in relation to particular disease, such as pateurellosis (Singh, 1948). But systematic data on the microflora in the different segments of respiratory tract are far from complete.

For convenience the respiratory tract in animals have been divided into two parts for study of microbial flora:-

(1) The upper respiratory tract which consists of nasal passages, nasopharynx and the oropharynx including tonsils and (2) the lower respiratory tract consisting of larynx, trachea, bronchii, bronchioles and lung tissue.

Payne and Derbyshire (1963) showed that the tonsils, small intestine and lungs were the major parts for the entrance of pathogens. They felt that pharyngeal lymph nodes become infected from infected macrophages and arose from the tonsiller crypts. It is evident from the present work that the percentage of micro-organisms in nasal cavity is more than that in trachea and lungs. It looks reasonable because nasal cavity is exposed directly to the atmosphere and the body defensive mechanism takes care of m.o. by the time they reach trachea and lungs.

In the present study it is demonstrated that the bacterial population of nasal passages differs from that of nasopharynx. In the former it is more whereas in the latter it is less. With regard to bacterial population of nasopharynx it might be assumed that organisms, which are inhaled, are ingested by

tonsils which normally arrest their growth and usually destroy these organisms. They have been known since long as a portal of entry for microorganisms including Mycobacteria as reported by Griffith (1907) and several other workers.

Several workers Singh, loc. cit; Lynch (1949); Florent and God bille (1950) and Carter (1956) are of the opinion that Pasteurella species were the normal inhabitants of the respiratory tract of mammals and become virulent when the resistance of the host is altered. Another group of workers Collier and Rossow loc. cit believed that Pasteurellae were not the normal inhabitants of respiratory tract.

Gale and Smith (1958) did not believe Pasteurella as a primary pathogen because the organisms can be recovered from the upper respiratory tract of normal animals. Hoerlein et al(loc.cit) believed that the isolation of Pasteurella in the lungs is associated with the presence of typical broncho pneumonia and the affected animals may harbour more organisms than the normal ones in nasal cavity.

But Magwood et al., (1968) differ. There was no consistent association between large number of a particular M.O. in the nasal passage and the presence of Pneumonia in the animals under their observations. He isolated P.haemolytica from 11 out of 14 cases of pneumonia but at the same time in another herd he could not detect any sign of pneumonia though 9 out of 12 animals were the carriers of these organisms.

It is known that septicaemic pasteurellosis occurs during periods of stress which may be due to diseases or environments. Twentyseven isolates of P.multocida and six isolates of P.haemo-

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lytica were recovered from pneumonic as well as from apparently healthy parts of the respiratory tract of 9 cattle and 4 buffaloes. Organisms isolated from pneumonic lungs proved to be more pathogenic for mice and rabbits than those obtained from apparently normal organs. Out of 9 cattle six were calves which yielded Pasteurella species from their respiratory tracts. These calves were also infected with foot and mouth disease; which was prevalent from before in the farm since a fortnight. They had lesions of the disease on tongues and clefts of their hoof. Majority of the calves had pneumonic lungs.

So it was concluded that the outbreak started with foot and mouth disease virus which had the upper hand in causing mortality in the beginning while during latter stage Pasteurella took over as the secondary invader. It indicates that the P.multocida and P.haemolytica could be one of the important causes of pneumonic pasteurellosis of cattle, especially when they were in stress. It is evident from the present finding that when the resistance of calves was reduced due to continued viral infection Pasteurella took over as the main causative agent.

These findings correlate the report of Carter and Rowsell (1958) and Resinger et al (1958) in other diseases like viral pneumonia of calves which may be a common precursor of pneumonic pasteurellosis.

Out of 520 isolates obtained, 38 isolates of E.coli were recovered from 13 cattle and 26 isolates from 10 buffaloes. This indicated that 13.0% cattle and 16.4% buffaloes were normal carriers of E.coli in their respiratory tracts. Nangyalai (1969) showed carrier rate of E.coli in cattle 15.8% while Kumar isola-

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ted 22.5% E.coli from upper respiratory tract of calves. In the present study out of 65 adult cattle, 3 cattle (4.6%) yielded E.coli but on the other hand 10 calves (28.5%) out of 35 yielded E.coli. The result is quite close to Nangyalai and Kumar as stated above.

It is evident from Table VII that 27 typeable strains against 44 sera of "O" groups were isolated from 17 apparently healthy animals. In order of frequency they fell into groups 086, 09, 0119, 0115, 0126, 08, 026, 035, 041, 055 and 0127 while 21 typeable strains, recovered from dead animals, were distributed in ten "O" antigenic groups 0119, 0126, 026, 055, 015, 041, 078, 086, 0101 and 0127 in order of decreasing frequency. Serological groups 0119 and 0126 were more frequently recovered from dead animals while 086 and 09 were more frequently associated with apparently healthy cattle and buffaloes.

Similar results have been obtained by Ulendeev (1963) who reported frequent occurrence of these serotypes in sick as well as in normal healthy animals. In calves 0119 and 026 serological groups were frequently found in sick and dead calves while 086 was more prevalent among apparently healthy cattle. The serotype 078 was isolated from a dead calf.

These serotypes are known as pathogens for man (Taylor and Charter,1952; Smith, 1953; Kauffman, 1954). Taylor (1961) reported that 0119 serotype was more frequently encountered in an outbreak of infantile diarrhoea. The pathogenic serotypes 086, 026, 055 and 078 cause enteritis in calves. 086 is associated in human diarrhoea (Edward and Ewing, 1962).

Lovell (1955), Gay (1965), Sojka (1965) and Kolya Kov

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et al.,(1970) suggested that certain serological types of E.coli were associated with colibacteriosis in calves but majority of these serotypes were confined to strains isolated from septecaemic form of the disease. Serotypes 015, 026, 035, 086, 0115, 0117 and 0137 were also encountered. Such serological uniformity was not found in strains isolated from diarrhoea. Generalized Coli infection may not originate from the intestinal tract for it can also originate from tonsils and umbilicus. Nasopharyngeal route of infection has been demonstrated to be quite severe by Fey and Margadant (1960).

It is evident that E.coli can survive as saprophytes in soil and surroundings. These facilitate the potential sources of infection, especially to young ones. Once they established in the intestinal tract, the host becomes carrier. Under different situations these carriers can be a potent source of infection to others depending upon the virulence of organisms and host cells resistence.

Klebsiella. pneumoniae has been recorded in respiratory tract of about 5.0% normal individuals (Bloom field,1921) and occurred as secondry invader in the lungs of patients with bronchielacsis, influenza and tuberculosis (Bloomfield loc cit). However, Hyde and Hyde (1943) described it to be the primary cause of pneumonia in less than 3.0% of all bacterial pneumonia.

In the present finding one isolate was discovered from a bullock which was also harbouring M.bovis. It seems that it was simply a secondary invader in this case.

Salmonella occurs in young and adult ones whose equilibrium become altered by intercurrent infection. This facilitates the

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multiplication of these organisms in intestine and ultimate invasion of blood stream. The resulting septicaemia may be fatal. At this stage of the disease, if someone survives, localisation of the Salmonellae occurs in one or more internal organs. Animals (men) become chronic carriers, excrete Salmonella from galbladder and intestinal wall into faeces and occasionally in milk. Intestinal involvement is usually absent in adult and may produce local abscesses in the perineal and pelvic region, cholecystitis, pericarditis, pneumonia etc.(Blood and Henderson, 1963). In the present study out of 100 cattle, 4(4.0%) yielded Salmonella from lungs and one of these four from trachea also. None of the nasal swabs yielded Salmonella. The occurrence of Salmonella in buffaloes was nil. All the isolates were recovered from pneumonic lungs except one from trachea of adult cattle. All these lungs showed various degree of hepatisation.

The antigenic characters of all the isolates were identified as 9,12: g,m. Bosworth loc cit isolated S.dublin from calves (suffering from pneumonia) with antigenic structer 1,9,12: g,p. Thomos loc cit found that S.dublin and S.enteritidis were equally prevalent in cattle.

Cattle, sheep, goats and birds are the carriers and excrete these organisms, their primary site of colonisation being the gasterointestine. They remain viable for a period in the polluted material.

During the present study absence of these m.o. in the nose indicates that these organisms are not normal inhabitant of the upper respiratory tract. Most probably they invaded lung tissues from foci in the intestinal tract and gave rise to pneumonia.

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Out of 100 cattle examined, 5 (5.0%) showed Streptococci in their respiratory tracts while 2 (2.7%) out of 73 buffaloes yielded Streptococci. Altogether 13 isolates were recovered. Of these 8 isolates were from cattle and the remaining 5 were from buffaloes. All isolates were beta-haemolytic. The data of the present findings confirm the findings of Kumar loc cit and Nangyalai loc cit.

Biochemical characteristics of one isolate from cattle was similar to those of group B of Lancefield; two like subgroup b of group C of Lancefield and five were like subgroup c of group C of Lancefield whereas isolates from buffaloes were equally divided biochemically in subgroups b and c of group C of Lancefield. The incidence of Streptococci varies considerably (Obiger loc cit). Daleel and Frost loc cit recovered 66.8% of Streptococci commonly incriminated in mastitis while 16.5% were enterococci. Obiger loc cit and Fornani, (1958) isolated Streptococci from tonsils of cattle while Dhanda (1955) isolated the same m.o. from the abscesses in the lungs of a calf.

Strep. dysagalactiae and Strep. uberis are associated with mastitis. According to Daleel and Frost in dairy cows haemotogenous infection of the damaged udder tissue by m.o. from tonsils is an explanation of such disease. They are also of the opinion that the tonsil works as a reservior of this organism. According to McCarty (1954) this m.o. has a special predilection for the lymphatic system and for the pharynx.

The primary site of colonisation is the nose and so these m.o. may be shed in droplets by coughing and may give rise to infection to ther animals or man. Magwood et al., loc cit were

of the opinion that beta haemolytic Streptococci together with Pasteurella and Micrococci constitute the basal flora of upper respiratory tract whereas Burrow (1959), Donal and Mann (1950) Hammer (1956) have recorded Streptococci as potent pathogens for respiratory ailments of cattle and sheep.

The findings obtained during the present study have confirmed some of the results of earlier workers indicating the importance of group B and C. Streptococci of Lancefield.

Thirtyfive (35.0%) out of 100 cattle and 19 calves out of 35(42.9%) yielded Staphylococci. Altogether 93 isolates were recovered from the respiratory tract of cattle. Of 38 (40.8%) coagulase positive isolates, 31 (81.5%) produced gelatinase. While 45 isolate out of 55 which were coagulase negative produced gelatinase. From buffaloes 56 isolates were recovered. Of these 21 (38.5%) were coagulase positive and the rest were coagulase negative. A total of 22 isolates (39.3%) were beta haemolytic and 16(76.1%) of these produced gelatinase. A total of 25 isolates produced golden pigment and were beta haemolytic. Organisms producing lemon yellow pigment were coagulase negative and non haemolytic. They failed to liquefy gelatin. Pgment production was similar for both buffaloes and cattle. According to Baired and Parker (1965) coagulase test can easily be employed in screening organisms of this group because coagulase has been implicated as a factor in pathogenicity . It is evident from the Table IV that coagulase positive strains were found in and around the nares and were more frequently recorded in pneumonic lungs. On the other hand the coagulase negative strains were unevenly distributed.

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The role played by nose as carrier, colonisation and transmission required many intreguing difficulties for assessing the pathogenicity of Staphylococci infection in animals. Some are persistent carrier while some are intermittent and some mobilise all attempts to rule out the Staph to become established in nasal mucosa.

In the present finding the occurrence of high incidence of Staph. aureus in dead calves might have been due to ingestion of infected milk from cows harbouring these organisms in their udders, most probably due to suffering from mastitis as Staph. aureus is an established causative agent for this disease.

Members of the genus Corynebacterium have been isolated from six (8.2%) out of 73 buffaloes examined during this study.

In addition, five (10.0%) isolates were made from nose of buffalo calves. Seven (23.3%) out of 30 dead calves yielded Corynebacterium equi (C.equi) in their lungs.

As recorded earlier Francies, Giedel and several other workers have recorded C. pyogenes in tonsils of normal cattle.

Seven isolates of C.equi were isolated from the pneumonic lungs of 7 dead calves which were previously affected by F.M.D. virus. When the outbreak of F.M.D. was subsiding Pasteurella took over the weak calves along with Corynebacterium as secondary invaders, most probably, because of stress caused by F.M.D. virus.

C.equi might be present at that time on the superficial tissue and became established causing damage of the host defensive mechanisms. Mallick and Singh (1968) and Holtman (1945) isolated C.equi from the lungs of calves. Their results have

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been confirmed by this study. C.pyogenes was isolated from the epiglottis of a calf showing diptheratic necrosis. Similar isolation was made by Kumar (1965).

In addition, members of genera Bacillus and Pseudomonas were isolated from the respiratory tract of cattle and buffaloes. Bosworth and Lovell loc cit isolated haemolytic Coccobacilli from the nasal cavities of goats, cattle, and sheep suffering from nasal catarrh.

Puscher and Toepfer (1967) also isolated Pseudo.aeruginosa from 63 slaughtered calves. The commonest lesion in affected calves (40.0%) was pneumonia. Winter and Conner (1957) also recorded Pseudomonas from the lungs of a cow.

In the present study Pseudo. aeruginosa was recovered from the nodules and absesses of lungs of 2 dead calves (6.6%) out of 30 calves examined. These m.o. might have accelarated the death of these calves acting as secondary invaders.

From the nares of 2 buff calves which were suffering from nasal catarrh Pseudo. aeruginosa were also isolated. Azizuddin and Nair (1954) also recorded Pseudomonas from the abscesses of lungs of buffaloes.

Mycotic pneumonia has been reported by several workers such as, Romberg (1960) Runnel et al.,(1964) Narain et al(1964) and recently by Damoderan.

In the present study, 3(3.0%) out of 100 cattle yielded fungi of the genus Aspergillus. The affected lungs showed various degrees of pulmonary lesions mostly nodular broncho pneumonia. As aspergillosis of the respiratory tract appears often to be a complication of some other debilitating disease, the isolation

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of members of the genus Aspergillus from cows which had suffered from other complication is noteworthy.

In order to assess the incidence of T.B. in cattle attempts were made to collect suspicious lesions from lungs of cattle and buffaloes slaughtered in the local abbatoir. Nine cattle yielded M.bovis.

As even a few viable T.B. bacilli may lead to progressive type of tuberculosis, isolation of these organisms from cases of cattle used for human consumption is very important from the public health point of view. It is the characteristic for T.B. that healing and progressive lesions may coexist in the same organ. It indicates that in each focus of infection a process goes on, influenced in part by systematic and in a part by some local factors.

It is interesting that the incidence of tuberculosis bacilli in the cow and female calf is more than male. Saroop (1952) also found a high incidence of T.B. in female calf than in male. Chander Sekheran and Nair (1969) reported a case of T.B. in six months old calf. In majority of the cases examined the lesions were more confined to lungs. Grossly they were enlarged and contained white caseated nodules, 1-2 cm in diameter distributed throughout the parenchyma.

Francis (1958) reviewing pathgenisis of tuberculosis said that the dose of T.B.bacilli required to produce the infection by alimentry route was million times greater than by the respiratory route. These cases of tuberculosis in cattle might has been examples of infection through the respiratory tract.

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The incidence of T.B. in 9 cattle out of 100 examined denotes that cattle slaughtered for meat purpose are not only debilitated but carriers of potent pathogens. This needs proper inspection of animals before and after slaughter otherwise more people may be affected with this disease as the percentage of T.B. infection in human is already quite high in India.

The results indicate that the flora of m.o. is an ecological entity in which the bacteria interact with the host in a dynamic relationship. The bacterial population may be fluctuating according to the environmental factors in which the host resides.

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SUMMARY

1. During the course of present study and investigation 551 samples were collected from nose, trachea and lungs of 173 cattle and buffaloes of different age groups for the isolation of microorganisms inhabiting these organs.

2. A total of 520 isolates were recovered from 551 samples.

3. The percentage of various m.o. from cattle was recorded as follows:-

Staphylococci	35.0%
Bacilli	19.0%
Corynebacteria	14.0%
Escherichia	13.0%
Pasteurella	9.0%
Mycobacteria	9.0%
Proteus	7.0%
Streptococci	5.0%
Pseudomonas	5.0%
Micrococci	5.0%
Salmonella	4.0%
Klebsiella	4.0%
Unidentified Gram negative rods	4.0%
Fungi	3.0%

4. The frequency of isolation of various m.o. from buffaloes is as follows:-

Staphylococci	35.6%
Bacilli	20.6%
Escherichia	16.4%
Corynebacteria	8.2%
Pasteurellae	5.5%
Proteus	5.5%
Pseudomonas	4.1%
Micrococci	4.1%
Klebsiella	2.7%
Streptococci	2.7%
Unidentified Gram negative rods	9.5%

5. The carrier rate of Pasteurella among cattle 9.0% as against 5.5% in buffaloes. Six isolates of P.haemolytica were divided into two biochemical strains whereas 27 isolates of P.multocida were grouped into four biochemical strains on the basis of their physiological and biochemical characteristics.

Pasteurella which might have been a commensal worked as a potent pathogen after the animals lost their resistence due to F.M.D. infection.

6. Important physiological characters such as pigment production, coagulase activity, haemolysin and gelatinase activities of Staph isolates were extensively studied. In cattle, the percentage of coagulase positive isolates was 40.8% against 47.6% found in buffaloes. All strains (except 3 isolates) producing golden pigment and some of the colourless colonies

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were coagulase positive and haemolytic and most of them produced gelatinase while lemon yellow pigment producing cells were neither coagulase positive nor haemolytic except that some were gelatinase positive.

7. About 13.0% of cattle were natural carriers of E.coli as against 16.4% buffaloes. Serotypes 0119 and 0126 were predominant in dead calves while 086 and 09 were quite common to healthy cattle and buffaloes, serotype 078 which is considered as a virulent serotype, was isolated from the lungs of a dead calf.

8. Five isolates of Salmonella enteritidis (9,12: g,m.) were recovered from lungs of adult cattle showing various degree of hepatisation. No Salmonella was isolated from buffaloes.

9. Five percent cattle were carrier of Streptococcus belonging to Lancefield's groups B and C whereas 7.0% buffaloes carried Streptococcus subgroup b and c of group C of Lancefield.

10. Fourteen per cent of cattle were found to be carrier of Corynebacterium as against 8.2% buffaloes. C.pyogenes and C.equi were the only species isolated.

11. 5.0% cattle were the carriers of Pseudomonas as against 4.0% buffaloes.

12. Three isolates of Aspergillus were recovered from 3.0% cattle as against none from buffaloes.

13. Nine cattle were found to be infected with M.bovis. This organism was not isolated from any of the buffaloes examined.

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BIBLIOGRAPHY

Azizuddin, I.M. and K.P. Chandrashekheran Nair. 1954. Studies on Pseudo. aeruginosa infection in animals. Madras Vet. Coll. Ann., 12 : 17- 22.

Ainsworth, G.C., and Austwick. 1959. Fungal diseases of animals. Review series No.6- Commonwealth Agriculture Brireaux, Farmhan Royal Buck England.

Augus, K.W., and N.J.L. Gilmour.1970. The occurrence of mycotic lesions in calves experimently dosed with M.avium. J.Comp. Path., 80 : 187-193.

Baird Parker, A.C. 1965. A classification of Micrococci and Staph based on physiological and biochemical test. J.Gen. Microb., 30 : 409-427.

Belchev, D. et al., 1967. Studies on aetiology of enteritis in new born calves in Bulgaria. Vet. Med. Nauke.Sofy. No.5 pp. 43-56.

Blood, D.C., and J.A. Henderson. 1963. Veterinary Medicine. A text book. 2nd edition. p. 466 and 477.

Bloomfield., A.L. 1921. Amer. Rev. Tubec., 3 (91), 55.

Bosworth, T.J.., and R.Lovell. 1931. Second. Rep. Inst. Animal path. Univ. of Cambridge. pp. 21-27 (Cited by B.K. Tiwary A. thesis. M.U.)

Bosworth, T.J., and R. Lovell. 1944. J.Comp. Path., 54 : 168.

Breed, R.S., E.G.D. Murray., and N.R.Smith. 1947. Bergey's Mannual of determinative bacteriology. Seventh edition. The William, and Wilkin, Co., Baltimore.

Burrow, W. 1959. Text book of microbiology. Seventh edition. p. 423-425.

Bythell, D.W.P. 1945. Bovine Pasteurellosis. The occurrence of haemorrhagic septicaemia and broncho pneumonia simultaneously in one outbreak among young calves. Vet. Rec., 57 : 289-290.

Carter,G.R. 1956. Some remarks on shpping fever in Canada. Canad. J. Comp. Med., 20 : 289-291. French summary in vet. Bull., 27 : 6 27 : 675.

Carter,G.R., and H.C. Rowsell. 1958. Studies on pneumonia in cattle. II An enzootic pneumonia in calves in Canada. J. Amer. Vet. Med. Ass., 132 : 187-190.

Chandrashekeran Nair, K.P. and R. Ramkrishnan. 1969. Pulmonary T.B. in a calf. Indian J.Anim. Hlth. 8 : 69.

Christensen, W.B. 1946. J. Bact., 52 : 461.

Collier, J.R., W.W.Brown. and T.L.Chow. 1962. Microbiological investigations of natural epizootics of shipping fever of cattle. J. Amer. Vet. Med. Assoc., 140 : 807 -810.

Collier, J.R. and C.F. Rossow. 1964. Am. J.Vet. Res., 25 : 291. Cited by Singh and Mallick. 1968. Indian Vet. J. 45: 565-571.

Cordes, D.O., D.C. Dodd. and O.Hare. 1964. N.Z. Vet. 12 : 101.

Cruickshank, Robert. 1969. Medical Microbiology. 11th edition. English language book society and E. & S. Living Stone, Ltd.,

Daleel, E.E. and A.J.Frost. 1967. Some observations on the bacterial flora of the bovine tonsil. A thesis for M.V.Sc., under a Commonwelth of Australia's scholarship. Br. Vet. J., 123 : 232-236.

Davies, G. 1967. Isolation of Mycoplasma from calf lungs. J. Comp. Path., 77 : 353- 357.

Davis, D.J. 1923. J. Am. Med. Ass., 81 : 1172. Cited by Daleel and Frost 1967.

Dhanda, M.R. 1955. Isolation of Strept. agalactiae from the abscesses in the lung of a calf. Indian J. Vet. Sci., 32:40.

Diwvedi, J.N. and C.M. Singh. 1966. Pulmonary Tuberculosis in buffaloes. Indian Vet. J., 43: 582-585.

Donald, L.G. and S.Q.Mann. 1950. Streptococcus pneumoniae infection in calves. Vet. Rec., 62 : 257-258.

Dubos, R.J., and Middle brook, G. 1948. Cytochemical reaction of virulent tubercle bacilli, Am. Rev. Tuberc. 58: 698-699

Dubos, R.J. and J.G. Hirsch. 1966. Bacterial and Mycotic infections of Man. A text book. Asian edition pp 377 and 616.

Edwards, P.R. and W.H.Ewing. 1962. Identification of Enterobacteriacease. 2nd edition. Burgees publishing Co., Minnesota, U.S.A.

Eggert, M.J. and P.F. Romberg. 1960. Pulmonary aspergillosis in a calf. J. Am. Vet. Med. Ass., 137 : 595.

RAJENDRA AGRICULTURAL UNIVERSITY, M. Sc. (Vet.) THESIS 1971

Elliot, L.P. 1966. Diss. Abst., 26 : 5055- 5655. Cited by Nangyalai, M.A. 1969. A thesis Magadh University.

Flatla, J.L.1943. Vet. Bull. 13 : 312 (Cited by Nangyalai,M.A. 1969).

Florent, A. and M. Godbille. 1950. Infection in animals in Belgium caused by a haemolytic Pasteurella and penicillin sensitivity. Ann. Med. Vet., 94 : 337-361 Vet. bull, 21:28.

Fornani, Q. 1958. Veterinariya Ital., 9 :201.

Francis, J. 1941. A bacterial examination of bovine tonsils and vaginas., 97 : 243.

Francis, J. 1958. T.B. in animals and in man. Cassell, Co., London. pp. 357. Cited by Clay A.L. in Aust. Vet. J. of 1971.

Gale, C. and H.R. Smith. 1958. Amer. J. Vet. Res., 19 : 815

Gay, C. C. 1965. Bact. Rev., 29 : 75-101.

Geidel, E. 1952. Inaugural Dissertation, Munich. Cited by Daleel and Frost 1967, M.V.Sc., thesis.

Gourlay, R.N. 1969. Isolation of Mycoplasma like m.o. from pneumonic lungs of calves. Vet. Rec. 84 : 229-230.

Gourlay, R.N., A.Mackenzie and J.E.Cooper. Studies of the microbiology and pathology of pneumonic lungs of calves. J.Comp. Path. 80 : 575-584.

Griffith, A.S. 1907. T.B. in animals and man, a study in comprative Path., pp. 303-304. London Cassell.

Gross, W.B. 1958. Amer. J. Vet. Res., 29: 224.

Hajek, V. and Marsalek,E. 1970. A study of Staph isolated from upper respiratory tract of diff. animal species. III Physiological properties of Staph aureus strain of percin origion. Zentble Bakt. Paresit Kde. 1 (orig)., 214 :68-74.

Hamdy, A.H. and A.L. Trapp. 1959. Investigation of nasal microflora of feed lot calves before and after weaning. Am. J.Vet. Res., 28 : 1019-1025.

Hammer, D. 1954. The occurrence of Strep. pneumoniae in calves, with reference to epidemiology and symptoms. Zbl. Bakt.1 (orig). 161 : 269-279. (Cited in Vet. Bull. 26:3).

Hamnel, P.H. 1970. Isolation of P.multocida Carter type E in Tanzina. Vet. Rec., 86: 42-43.

RAJENDRA AGRICULTURAL UNIVERSITY, M. Sc. (Vet.) THESIS 1971

RAJENDRA AGRICULTURAL UNIVERSITY, M. Sc. (Vet.) THESIS 1971

Harcourt, R.A. and F.G.A. Thompson. 1969. Mycotic abortion and mycotic pneumonia in a cow . Vet. Rec., 85 : 199-200

Hoerlein, A.B., S.P.Sexena and M.E. Mansfield. 1961. Studies on shipping fever of cattle II Prevalence of Pasteurella species in nasal secretion from normal calves and calves with shipping fever. Amer. J. Vet. Res., 22 :470. Cited by Magwood et al., 1969.

Holtman, D.F. 1945. J.Bact., 49 : 159-162. Cited by Nangyalai 1969. A thesis M.U.

Hormachae,E and N. Peluffo. 1959. Bull. W.H.O., Org. 21., 2:247.

Jensen, K.A. 1955. Second report of the subcommittee of laboratory methods of the International Union against Tuberculosis. Bull. Int. Un. Tuberc., 25 : 89.

Katikaridis, A. 1969. The Mycobacteria responsible for T.B. among cattle slaughtered at Munich. Inaug. Diss. Tierzt. Fab., Munichen. pp 48. Cited in Vet. Bull. 40: 1.

Kauffmann, F. 1947. Journal Immunology., 57 : 71-100.
--1954. Enterobacteriaceae 2nd edn., Copenhagen.

Kolyakov, Ya. E., S.S.Gielson and L.S. Kavruk.1970. Kolibacterios Telyat, Moscow, Izdatael stvokolos. Cited in Vet. Bull. 41 in editorial note of March,1971.

Kubica, G.P. and R.E. Beam. 1961. Amer. Rev. resp. Dis.,83:733.

Lall, J.M., G. Singh., and B.R. Sengupta.1969. Incidence of T.B. among cattle and calves in India. Indian J.Anim. Sci., 39 : 51-58.

Laybourn, R.L.1924. A modification of Albert's stain for the Diptheria bacillus. J. Amer. med. Assoc., 83:121.

Leach, R.H. 1970. The occurrence of Mycoplasma arginini in several animal hosts. Vet. Rec. 87, 8: 319-320.

Leib, E. 1951. Inaugural Dissertation Munich.

Leisslie, I.W. and K.J. Birn.1967. T.B. in cattle caused by avian type. Vet. Rec., 80: 559-564.
--1970. M.avian infection in cattle and pig in Gr. Britain, Tubercle London; 51: 446-451.

Lynch, T.1949. Pasteurellosis in calves in Ireland. 2 cases of acute haemorrhagic septicaemia. Irish Vet. J., 3 : 290-292. Cited in Vet. Bull. 21: 27

//Magwood, B.E., D.A. Barnum and R.G. Thomson.1969. Nasal bact. flora of calves in healthy and in pneumonic prone herds Can. J. Comp. Med., 33 : 237- 243.

Marks, J. and D.R. Trollope. 1960. Tubercle, London., 41 : 51, 127, 133.

McCarty, M. 1954. The antibody response to streptococcal infections in McCarty, M.(ed.): Streptococcal infections, pp. 130-142. New York, Columbia Univ. Press.

Mobbs, 1931. Ann. Rep. Vet. Res. Inst. Mukhteshwer. Cited by Nangyalai,1969, a thesis M.U.

Mohan, K., M.N.Sinha., R.P.Singh and C.M.Gupta.1968. A study of immunity against P.multocida in buff calves and their carrier status. Vet. Rec., 83: 155-156.

Munday, B.L.1967. Mycotic infection in cattle.N.Z. Vet. J., 15: 149. Cited in Vet. Bull. 38; 1321.

Nangyalai,M.A. 1969. Aerobic bact. flora of Resp. tract in calves and goats. A thesis Univ. Magadh.

Narayana, J.V.., P.R.Rao., J. Christopher and G.A. Shastry.1964. Pulmonary aspergillosis in murrah buffalo. Indian Vet. J., 41 : 523-525.

Newson, I.E., and F. Gross.1932. Some bipolar organisms found in pneumonia in ship. J.Amer. Vet. Med. Assoc., 80:711-719.

Obiger, G. 1962. Z. Hyg. Zool., 148, 405. Cited by Daleel and Frost in 1967, a thesis Univ. Austra.

Omar, A.R., Cheah. P. Phay and S.C.Shanta.1962. The isolation of a virulent strain of P.multocida from an apparently healthy buffalo. Brit. Vet. J., 118,: 71-73.

Payne, J.M., and J.B. Derbyshire.1963. Portals of entry for bacterial infection in calves and piglets with particular reference to the tonsil. J.Path. Bact., 85: 171-178.

Pilot, I., C.Buck. and D.J. Davis. 1936. Proc. Soc. exp.Biology Med., 34: 233. Cited by Daleel and Frost 1967. Brit. Vet. J., 123 : 232-236.

Prasad, L.B.M. 1965. Personnel communication. Cited by Nangyalai 1969, a thesis M.U.

Pulverer,G. and H.J.Entel. 1967. Physiological occurrence of coagulase +ve Staph. in the animal kingdome.1. Occurrence in the nasopharynx of domestic and wild animals II Differentiation of strains from the nasopharynx of healthy animals. Zentb. Bakt. Parasit Kde.1 (Orig.) 201: 344-363.

Purohit, A.K. and P.N. Mehrotra. 1969. Incidence of T.B. in cattle in Rajasthan. Indian Vet. J., 46: 5-12.

RAJENDRA AGRICULTURAL UNIVERSITY, M. Sc. (Vet.) THESIS 1971

Puscher, J. and H. Toepfer. 1967. Occurrence of Pseudomonas. aeruginosa in slaughter animals. Tierarztl Unesch., 22: 611-612 and 615-617.

Rathore, B.S. and N.P.Singh. 1969. Pulmonary T.B. in buffaloes. A study on its incidence and pathology. Indian J.Anim. Hlth. 8 ; 85-87.

Reisinger, R.C. et al., 1959. J.Amer. Vet. Med. Assc., 135; 147. Cited in Blood and Henderson 1963. Vety Medicine.

Robberts, R.J. 1969. Isolation of Corynebacterium haemolyticum from a case of ovine pneumonia. Vet. Rec., 84, 490.

Rude, T.A. Jr. 1963. J.A. V.M.A. 142; 751. Cited by Tiwary, B.K. a thesis M.U.

Runnels, R.A., W. S. Moulux and A.W.Moulex. Principles of Vety. Path. 7th edn. The Iowa State University Iowa Press Anes.

Schenk, H. 1938. Vet. Bull. 12: 74. Cited by Nangyalai, 1969. A thesis M.U.

Schimid, G. 1933. Vet. Bull., 4: 207. Cited by Tiwary B.K. 1969. A thesis M.U.

Schipper, I.A. 1963. Vet. Med., 58: 731. Cited by Nangyalai, 1969.

Schobesch, O; C.Dragchici and T.Ciuclea. 1970. Value of colonisation of the nasopharynx of new born calves with apathogenic bact in the prophylaxis of digest and resp. diseases. Preliminary note. Revta Zooteh. Med. Vet. Bucurestic., 2: 45-49.

Silberg, S.L., H.M. Pairish and D.C. Blender, 1967. Relationship between type of illness and asymptomatic Staph nasal infection in animals. Vet. Med. small animal., 62: 861-863.

Singh, Gurkipal and D.T. Pernaik. 1965. Indian Vet. J. 42; 159-168.

Singh, N. 1948. Indian J. Vet. Sci., 18: 77.

Singh, N.B. and B.S.Mallick. Microflora of the Resp. bact. of buffaloes. 1 Isolation of bacterial agents. Indian Vet. J. 45: 565-571.

Smith, J. 1953. J.Path. Bact., 66; 503.

Sojka, W.J. 1965. E.coli in domestic animals and Poultry. Commonwelth Agricultural Bureau, Farnhan, Royal Buxk, England.

RAJENDRA AGRICULTURAL UNIVERSITY, M. Sc. (Vet.) THESIS 1971

Sroop, 1955. Cited by Prohit & Malhotra, 1969. Indian Vet.J., 46 : 5-12

Stors, J.1968. Psittacosis lympho granuloma agent in bovine pneumonia. J.Am. Vet. Med., Assoc., 152: 814-819

Sudakov, N.V.1968. Typing of Klebsiella strains isolated from animals. Veterinariya Moscow. 9: 22-24.

Sudaric, F. 1965. 18 : 229-230.

Taylor, J. 1961. Infantile diarrhoea and vomiting, clinical & bacteriological investigation. J. Appl. Bact. 24: 316.

The society of American Bacteriologist. 1957. Manual of microbiolo-methods. McGraw- Hill Book Company. Inc., New York.

Thomas, J. 1961. Am. Med. Vet., 105 : 206

Thomson, R.G., M.L. Benson and M.Savan.1969. Pneumonic pasteurellasis of cattle. Microbiology and immunology. Can.J.Comp. Med., 33 : 194-206

Tiwary, B.K.1969. M.V.Sc., thesis Univ.Magadh.

Tuboly, S. and I.Szalio. 1967. Isolation of Mycobacterium. tuberculosis Vaccae from cattle. Acta. Vet. Hung., 17 : 149-151.

Ubertini, B. 1939. Clin. Vet. Milano., 62 : 6-11.

Ulendeav, A.I. 1963. pathogenic serotypes of E.coli associated with acute gastero intestinal disease of calves. Veterinaiya, Moscow, No.5 pp. 15-18 (R).

Viallier, J. and R.M.Caryre.1966. Performance of the identifying characteristics of bovine T.B. bacilli. Bull.Soc.Sc. Vet.Lym. 68: 203-06. Cited in Vet. Bull. 37; 1155.

Wang, C.T., M.H. Wu., Y. Mathari., F.Abate and A.Tarema.1969. An O.B. of H.S. of Cattle in Georgia & Gurmba Begenbder procince Ethiopia. J.Taiwan Assn. Anim. Hlth. Vet.Med., 14:44-59.

Weisman, G.E. and G.Hilker. 1968. Characterisation of P.haemolytica isolated from resp. tract of cattle. Can.J.Comp.Med., 32 : 498-504

Wijewanta, E.A. and K.C. Kurunaratin. 1968. Studies on the occurrence of P.multocida in the nasopharynx of healthy cattle. Cornell Vet., 58 : 462 - 465.

Wilson, G.S. and A.A.miles.1966. Topley & Wilson's principles of bacteriology and immunity, 5th edition, pp. 554.

Wilson, M.R.& R.G.Thomson. 1968. Chlamydia pneumonia in calves. Res. Vet. Sc., 9 : 467-470.

Winter, H. and R.F.C. Conner. 1957. Aust. Vety. J. 33 : 83.

RAJENDRA AGRICULTURAL UNIVERSITY, M. Sc. (Vet.) THESIS 1971

Zagaevskii, L.S. 1962. Veterinariya Moscow No.9, P. 15.

Zorawki, C. 1969. Some biological cytochemical & biochemical properties of acid fast bacilli isolated from cattle. Polskie Archom Wet., 11 : 647-658.

RAJENDRA AGRICULTURAL UNIVERSITY, M. Sc. (Vet.) THESIS 1971