MICROBIOLOGICAL STUDY OF EXTERNAL EAR OF DOGS AND BUFFALOES IN HEALTHY AND IN DISEASED CONDITION (OTITIS EXTERNA) IN BIHAR WITH SPECIAL REFERENCE TO ANTIBIOTIC SENSITIVITY TEST " IN VITRO "

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This is to certify that the work embodied in this Thesis entitled "MICROBIOLOGICAL STUDY OF EXTERNAL EAR OF DOGS AND BUFFALOES IN HEALTHY AND IN DISEASED CONDITION (OTITIS EXTERNA) IN BIHAR WITH SPECIAL REFERENCE TO ANTIBIOTIC SENSITIVITY TEST "IN VITRO" is the bonafide work of Dr.Prem Chandra Verma and was carried out under my guidance and supervision.

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CERTIFICATE

Certified that the research work incorporated in this Thesis have not been published in part or in full in any other journal.

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INTRODUCTION

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

Diseases of the external ear are among the most common conditions observed by veterinary and medical practitioners. Among the domestic animals, mostly dogs and buffaloes are found to suffer from otitis externa. These two species of animals are also very much associated with the life of human beings. Though the disease does not prove fatal, its importance cannot be ignored from the public health point of view. Besides, it gives constant irritation and annoyance to animals which suffer from the disease.

The external auditary canal provides an ideal environment for bacterial and fungal growth because of the presence of moisture, accumulation of shed epithelial cells, cerumen and other debris.

Otitis externa is an acute or chronic inflammation of the external auditary meatus, characterised by pruritis and foul discharge. It is frequently unilateral but in number of cases both ears are involved. Adult and old animals are most commonly affected (Fraser, 1965).

The etiology of the disease is not clearly known. There are various predisposing factors : these include dirt, presence of ear mites, skin diseases, endocrine imbalance, moisture, foreign bodies, trauma, tumour, anatomical conformation of the ear and a number of generalised infections such as, distemper. Whatever may be the etiology of otitis externa there

is always an involvement of bacteria and fungi (Fraser,1961).

The normal flora of the ear canal in man and those found when otitis is present have been widely studied, but the microbiological study of the ear canal of normal and diseased domestic animals has not been extensively done (Grono,1967).

In India, no systematic attempt has been made to study otitis externa in domestic animals. Only scattered reports on the examination of a few cases are available.

The present study has been undertaken to isolate and identify various bacterial and fungal organisms associated with the otitis externa in dogs and buffaloes.

Attempts were also made to produce experimentally otitis externa with some of the isolated bacterial strains in apparently normal buffalo calves, dogs and rabbits.

In vitro sensitivity tests with some of the bacterial isolates were conducted for some of the common antibiotics used in the therapy of ear affections.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

1. History.

Otitis externa in dogs have been recognized since long. In 1887, Zurn and Plaut recognized that fungal infection of the external ear sometimes occurs as a cause of otitis externa. They isolated species of Aspergillus from inflammed external ear canal of dogs.

Hoffman (1898) described that chronic otitis externa occurred as a result of infestation with mites and other parasites, association with foreign bodies, blow or other unknown cause. Knowles et al. (1948) described 89 cases of canine otitis externa. Dam (1952) described 17 cases of the disease in dogs. Serth (1954) examined 27 cases of otitis externa in dogs and ten cases in cats and subjected them to bacteriological examination. Jones (1955) has given a preliminary report of the flora of external ear of dogs in health and disease. Gustafson (1955) for the first time carried out a systematic study of canine otitis externa in Sweden. Fraser (1961) examined 100 clinically affected dogs, 35 healthy and 351 unspecified cases of otitis.

2. Incidence.

(a) Incidence according to the breed of the animal : - Incidence of otitis externa in dogs is related to the breed of animals. Acute type of the disease is usually detected in

sensitive breeds e.g. miniature poddle; chronic type of otitis is generally found in docile breeds (Cross, 1962). Berg (1951) examined 5751 dogs with erect, semi-erect with dense hair and pendant ears. The incidence of otitis externa in these groups was found to be 4.2%, 13.3% and 7.1% respectively.

Joshua (1958) described that the incidence of otitis externa was higher in long drooping heavily feathered ears such as, the various spanial varities.

Grono (1969a) found out incidence of otitis externa in different breeds as shown in Table - I.

TABLE - I.

Incidence of otitis externa in different breeds

Breed	Number of dogs examined	Affected with otitis externa (%)
Cocker spanial	916	11.5
Labrador	326	8.0
Scottish terrier	125	8.0
Miniature poddle	124	7.2
Dachshund	484	4.5
Australian terrier	226	3.8
German shepherd	872	3.6
Scotch collie	319	3.1
Pomeranian	465	2.6
Kelpie	761	2.4
Fox terrier	916	1.7
Pekingese	275	1.5

Baxter and Lawler (1972) observed that the breeds showing higher incidence of the disease were cocker spaniel and Afgan (19% and 18%). Chihuahuas and terriers were the least affected (2% and 1%).

(b) Incidence according to the age and sex of the animals affected : - Tufvesson (1955) in an examination of 353 dogs found that most of the dogs with otitis externa were in the age groups of 5 to 8 years.

Fraser (1961a) investigated cases of otitis externa in dogs and observed that the mean age of dogs with otitis externa was 5.3 years in comparison to the mean age of healthy dogs which was 4.3 years. Grono (1969a) has given incidence of otitis externa in relation to age. The highest incidence (10%) was in the age group of 6 to 8 years and the lowest, that is, 2.0% in dogs under two years of age.

Tufvesson (1955) did not find any significant variation according to the sex of dogs. In his study of 353 dogs examined 41.8% of dogs with otitis externa were female as compared with 48.9% male. Fraser (1961) also did not find any evidence that the sex influenced otitis externa in dogs. The ratio of male and female dogs who suffered from the disease was almost the same, i.e., 1.65 to 1.00 in both otitis and normal population. However, Grono (1969a) found that the otitis externa in male was significantly higher than in female. According to his observation of 409 cases of otitis externa in dogs 265 were male and 144 were female.

According to Baxter and Lawler (1972) the cases of otitis externa were distributed almost equally between the sexes in both dogs (45 female and 42 male) and cats (16 female and 15 male). Of the dogs 13 were young (less than 1 year) and 74 were mature. All but two of cats examined were mature.

(c) Incidence according to season : - Grono (1969a) found that there was no significant seasonal variation in occurrence of otitis externa in dogs. The highest average monthly incidence was (6.4%) in December and lowest (3.6%) in June.

Baxter and Lawler (1972) found little seasonal difference in the incidence of otitis externa of dog and cat. Slightly more cases were seen in winter (May to October) but this was not significantly higher than in summer (November to April). A total of 55 and 58 per cent of dogs and cats respectively was seen suffering from this during six months of winter.

3. Etiology.

The exact etiology of otitis externa is not yet well established. Various factors have been recorded which play their role in production of otitis externa. The etiological agents so far observed by different workers may be divided under the following subgroups : (a) Bacterial agents, (b) Fungal agents, (c) Parasitic agents, and (d) Miscellaneous factors.

(a) Bacterial agents : - Bacteria have been implicated as the major cause of otitis externa in dogs by some investigators (Farrag and Mahmaud, 1953; Jones, 1955). Others contend that bacterial infection is usually secondary (Fraser et al., 1961).

The normal healthy external ear canal may be sterile, but Staphylococcus, Streptococcus and other commensals may be frequently present, which under suitable conditions, such as, trauma may become pathogenic (Ballenger and Ballenger,1943).

Dam (1952) isolated only staphylococci from 16 cases and Pseudomonas aeruginosa as well as staphylococci from one case of otitis externa in dogs. Farrag and Mahmaud (1953) while investigating a number of dogs suffering from otorrhoea found a high percentage of them infected with P. aeruginosa. Serth (1954) subjected 27 dogs with otitis externa to bacteriological examination. His findings are recorded in Table - II.

TABLE - II.

Micro-organisms isolated from otitis externa in dogs by Serth (1954).

Micro-organisms isolated	No. of cases
Staphylococcus	10
Staphylococcus + Proteus vulgaris.	2
Staphylococcus + Bacterium zopfi	1
Staphylococcus + Bacillus subtilis	1
Mucor mould	1
Mould (possibly Schizosacchraomyces)	1
Proteus vulgaris	2
Actinomyces	1
Pseudomonas aeruginosa	2
No organisms	6

Jones (1955) examined a total of 128 cultures taken from 78 dogs suffering from otitis externa. His findings are shown in Table - III.

TABLE - III

Micro-organisms isolated from otitis externa of 78 dogs by Jones (1955).

Micro-organisms	Ear		Total
	Left	Right	
Micrococcus pyogenes var. aureus	17	17	34
Proteus vulgaris	14	15	29
Proteus mirabilis	2	1	3
Micrococci	0	4	4
Enterococci	3	2	5
Micrococcus tetragenes	1	1	2
Streptococcus duran	1	1	2
Streptococcus faecalis	1	3	4
α-haemolytic streptococci	5	2	7
β-haemolytic streptococci	5	4	9
Non haemolytic streptococci	5	7	12
Pseudomonas aeruginosa	8	9	17
Alcaligenes faecalis	1	3	4
Escherichia coli	4	5	9
Klebsiella pneumoniae	1	0	1
Aerobacter aerogenes	1	3	4
Bacillus subtilis	7	9	16
Bacterium mycoides	0	1	1
Diptheroids	8	6	14
No growth	8	5	13

He concluded that Micrococcus pyogenes var. aureus, Proteus vulgaris and Pseudomonas aeruginosa were most prevalent.

Karl (1957) found that the most frequent micro-organisms found in the external ear canal are not only of genera Streptococcus and Micrococcus but also Proteus, Pseudomonas aeruginosa, E. coli and others.

Fraser (1958) pointed out that while Proteus and Pseudomonas occurred only in clinically affected ears, Staphylococcus and Pityrosporum sp , could be isolated almost as frequently from clinically normal ears as from abnormal ears. A higher proportion of staphylococci from affected ears coagulated rabbit plasma than those isolated from normal ears. Various micro-organisms isolated from normal and affected ears of dogs by him are shown in Table - IV.

TABLE - IV.

Micro-organisms demonstrated in otitis externa of dogs by Fraser (1958).

Species	70 healthy external ears of 35 clinically healthy dogs(%)	523 external ears of 363 dogs affected with otitis (%)
Staphylococci	54	61
Haemolytic streptococci	3	18
Non-haemolytic streptococci	30	25
Pseudomonas sp.	0	13
Proteus sp.	0	16
Coliform bacilli	6	13
Corynebacterium	16	17
Aerobic spore bacilli	44	42
Anaerobic spore bacilli	44	42
Pityrosporum sp.	36	44
Other yeast	4	6
Fungi	11	12

Grono and Frost (1969a) studied the microflora of 124 normal and 716 affected external ears of dogs. He concluded that both Pseudomonas and Proteus were significantly more prevalent in infected than in normal ear canal. His findings are recorded in Table - V.

TABLE - V.

Microflora of healthy and affected ears of dogs according to Grono (1969).

Organisms	From 124 healthy ears (%)	From 716 infected ears (%)
Staphylococcus aureus	47.6	30.9
Staphylococcus (coagulase negative)	74.2	8.0
β-haemolytic streptococci	0	7.4
α-haemolytic streptococci	15.3	5.2
Pseudomonas aeruginosa and other Pseudomonas	2.4	34.6
Proteus sp.	1.6	20.8
Coliform bacilli	42.7	7.3
Diptheroids	25.8	3.1
Bacillus sp.	74.2	8.5
Yeast	37.9	35.9
Aspergillus sp.	1.6	0.1
Streptomyces sp.	6.5	0.1
No growth	1.6	9.9

Wang (1972) analysed the cultures of 185 ears with otitis externa from 100 dogs and 159 normal ears from 87 dogs. He concluded that Staphylococcus aureus was the most important agent in purulent otitis externa. His observations are recorded in Table - VI.

TABLE - VI.

Micro-organisms isolated from healthy and affected ears of dogs by Wang (1972).

Micro-organisms	From 185 infected ears of 100 dogs		From 159 normal ears of 87 dogs	
	No. of ears from which isolated	%	No. of ears from which isolated	%
Coagulase positive staphylococci	123	66.4	24	15.0
Coagulase negative staphylococci	35	18.6	13	8.0
Proteus hauseri	34	18.0	Nil	
Pseudomonas aeruginosa	32	17.0	1	0.63
Haemolytic streptococci	28	15.0	23	15.0
Enterococcus faecalis	19	10.0	1	0.63
Candida ptropicalis	17	9.0	16	10.0
Non haemolytic streptococci	13	7.0	13	8.0
Other Pseudomonas	12	7.0	2	1.26
Alcaligenes	11	6.0	2	1.26
Klebsiella	10	5.0	Nil	
Micrococci	9	5.0	23	15.0

Baxter and Lawler (1972) made culture from 87 ears of dogs with otitis externa. Pityrosporum pachydermatis was the predominent species to be isolated, both as a sole organism and as a member of mixed flora. It was isolated fromatotal of 49 ears

which represents 56% of total number of cases from otitis. The most frequent associates of Pityrosporum pachydermatis in mixed flora was Staphylococcus aureus.

(b) Fungal agents : - Zurn (1887) observed that fungal infection sometimes occur in dogs. He isolated Aspergillus sp. from them. Jacob (1930) found Aspergillus, Mucor and Verticillium species from Otomycosis in dogs. Schoop (1951) was of the opinion that organisms of Blastomycetes group were important factor in canine otitis externa and he described the disease as blastomycosis as affected ears showed a profusion like blastomycosis. Ainsworth (1954) isolated Aspergillus fumigatus from 2.0% of the infected ears of the dog. Several workers have described a yeast Pityrosporum canis and are of the opinion that it plays an important role in the production of otitis externa in dogs (Gustafson, 1955; Joshua, 1958; Smith, 1968). Fraser (1961) studied the fungal flora of the ear canal of healthy and infected dogs. His findings are recorded in Table - VII.

He is of the opinion that the part played by Pityrosporum canis in initiating the infection is not obvious as their incidence in ear canal of both healthy and affected dogs is very similar. Wanger et al. (1968) reported otitis externa in dogs caused by Cryptococcus neoformans.

(c) Parasitic agents : - At least two ectoparasites are known to produce parasitic otitis. Otodectes cyanotis, the common ear mite of dogs and cats and Otobius megnini, the

TABLE - VII.

Fungal flora of ear canal of dogs according to Fraser (1961).

Name of organisms	Healthy dogs (70 ear canal)		Otitis in dog (400 ear canal)	
	Number	Per cent	Number	Per cent
Aspergillus.	4	6.00	13	3.00
Penicillium	3	4.00	12	3.00
Cladesporium	1	1.00	13	3.00
Greotrichum	1	1.00	8	2.00
Botritis	-	-	3	0.80
Candida	3	4.00	1	0.30
Rhodotorula	-	-	19	0.50
Pityrosporum	25	36.00	177	44.00

spinose ear tick. The parasites produce extensive irritation in the external ear canal. Kaufmann and Frost (1949) are of the opinion that otitis is often caused by mites (Otodectus cyanotis). In a study of 50 cases they found ear-itch in 28, i.e. in 56.0%. Grono (1969b) in a study of 350 dogs found 29.1% of them infected with mites and otitis externa was diagnosed in 24.1% of ears.

Various workers are of the opinion that the ear mite, Otodectes cyanotis, plays an important role in the etiology of otitis externa (McGinnis and England, 1949; Jennings, 1953; Koutz, 1955; Frost and Berisford Jones, 1958 and 1960).

(d) Miscellaneous factors : - There are various other factors responsible for causing otitis externa. Sutlie (1939) reported that foreign bodies, like presence of chaff, corn and bristles were the cause of otitis in as may as 13.0% of all the cases.

The anatomical conformation of ears in dog is said to be a predisposing factor in the prevalence of otitis in this animal. Krall (1936) was of the opinion that the collection of ear wax and poor ventilation of the external ear canal were the common cause of otitis. Kaplan (1951) mentioned dropping ears and lack of ventilation as being a predisposing factor. Joshua (1958) incriminated a particular conformation of external ear, for instance, heavily feathered ears, narrow type ear canal, coat type in wire haired breeds and thick skin type breed as the predisposing factor for the disease.

In young animals the otitis externa is frequently

associated with distemper (Brumley, 1950).

4. Clinical findings.

Otitis externa has been reported either as an acute or chronic condition. The acute condition as described by Cross (1962) is principally found in erect ear breeds, and miniature poddle and is characterised by intense pain, voilent shaking of head, anorexia and scratching at the affected ear. The discharge according to him is usually of a profuse and purulent type with a characteristic pale yellow colour whereas Fraser and his associates (1961) consider the discharge in acute otitis externa to be of dark colour.

Cross (1962) further describes the chronic type of otitis externa as extremely resistant to treatment. He is of the opinion that the reason for chronic type seems to be a combined bacterial infection and lack of ventilation due to long and heavy pendent ears. This condition is found generally in breeds of docile tempramant (Cockerspaniel, Beagle and Labrador) in which infection may go un-noticed for many weeks. Thus sufficient time is allowed for contamination with Proteus and Pseudomonas. The colour and type of discharge with the infection of Proteus and Pseudomonas is copious, tenacious, brown to red-brown material. But Fraser et al. (1961) considers the discharge in chronic otitis externa to be characteristically pale or light yellow in colour. According to them there is extensive prolifration of connective tissue and ulceration of ear canal in chronic otitis externa.

5. Experimental study.

Seibemnian (1889) for the first time started experimental studies to produce otitis externa in rabbits and dogs. He failed to produce fungal infection in the ear canal of these animals. Enlows (1935) was unable to produce otitis externa in the intact external ear canal of rabbits and guineapigs, with fungi. He was only able to do so after traumatizing skin severely. Salvin and Lewis (1946) were able to cause otitis externa in rabbits with a combination of trauma and infection. They were unable to produce clinical otitis with Pseudomonas alone. Witter (1949) was able to produce otitis externa in dogs with water, cottonseed soap, saponated solution of cresol and exudates from chronic otitis externa. Farrag and Mahmaud (1953) used 6 dogs for the experimental production of otitis externa. They instilled heavy saline suspension of Pseudomonas aeruginosa into the ears which were then rubbed vigorously in order to get the suspension in close contact with the skin. A slight inflammation was noticed in the ear canal of all 6 dogs after 5 days. After 10 days symptoms were more pronounced.Gustafson (1955) was able to produce otitis externa in dogs with Staphylococcus albus (toxin producing strain) and Pityrosporum canis. He failed to produce otitis with Pseudomonas pyocyanea in the intact skin. Grono (1969b) worked on the experimental production of otitis externa in dogs. His findings are shown in Table - VIII. The author used the various factors in different combination. The result obtained was variable.

TABLE - VIII.

Experimental production of otitis externa in dogs by Grono (1969b).

Agents applied for production of otitis externa.	No.of ear canal applied	Result (otitis externa produced)
Ear mites and Pseudomonas	3	2
Ear mites, Pseudomonas and trauma	3	3
Mites, sterile broth and trauma	3	1(mild)
Mites and other factors	18	9
Pure culture of Pseudomonas.	3	2
Sterile broth	3	1
Pseudomonas and trauma	3	1
Sterile broth and trauma	3	1(mild)

6. Otitis externa in dogs in India.

A few attempts have been made to study the microbiology of otitis externa in dogs and almost no attempt has been made to study the disease in buffaloes in India.

Azizuddin and Chandrasekhran Nair (1954) studied on Pseudomonas infections in animals. They found that Pseudomonas were also associated with the otitis externa of buffaloes and dogs. Singh and Rao (1959) studied 60 cases of otitis externa of dogs in Orissa. Of these cases 40 were treated medicinally

and 18 surgically. They made no attempt to isolate the causative organisms. Mathew et al. (1970) studied 30 cases of otitis externa in Bombay and isolated 52 strains of different micro-organisms from these cases. They also did sensitivity trial with furacin, terramycin and otoryl and found that Pseudomonas were resistant to furacin, terramycin and otoryl. In general most of the strains of micro-organisms isolated by them were sensitive to furacin.

Sinha (1970) isolated various types of bacteria and fungi from 200 healthy and 50 infected ear canal of dogs. Though Staphylococcus aureus, Staphylococcus albus, Streptococcus faecalis, Pseudomonas pyocyanea, E. coli and Klebsiella aerogenes were isolated from both normal and infected ears, the rate of isolation of Staphylococcus aureus and Pseudomonas pyocyanea was quite higher than other organisms from the affected ears. Proteus mirabilis, Proteus morganii and streptococci (β-haemolytic) were isolated only from infected ears. Micrococci and aerobic sporulated bacilli were present only in normal ears. Similarly, a large number of fungi were isolated from normal and affected ears but only Aspergillus niger and Pityrosporum canis was isolated in significantly higher rate from infected ears. He also studied with the experimental production of otitis externa in dogs. He was able to produce otitis externa with Staphylococcus aureus (100.0%) and Pityrosporum canis (50.0%) but failed to do so with Pseudomonas pyocyanea.

Dey et al. (1972) examined 21 ears having otitis externa and 10 normal ears for the presence of bacteria and fungi. Out of 19 isolates tested for antibiotic sensitivity test 14 isolates were sensitive to tetracyclines and streptomycin, 10 to chloramphenicol and six to penicillin.

MATERIALS AND METHODS

MATERIALS AND METHODS

(A) Selection of cases.

Materials from otitis cases for the present study were mainly collected from clinically affected dogs and buffaloes.

Dogs and buffaloes attending the Bihar Veterinary College Hospital for diseases other than those of the ear were taken as normal control. The materials from the clinically affected cases of otitis externa in dogs and buffaloes were collected from the following sources :

1. The Bihar Veterinary College Hospital, Patna.
2. The Government Veterinary Hospital, Bankipur.
3. The Government Veterinary Hospital, Muzaffarpur.

(B) Collection of material.

Ear swabs : - A copper wire measuring 8 to 9 inches in length with one end bent was used for swab making. Swabs were placed into clean test tubes and were sterilized at 170°C for 1 hour. The external ear was first cleaned thoroughly with water and dried with cotton wool. It was then swabbed with rectified sprit. After it was dry the sterilized swab was inserted into the ear canal taking care that the canal was not injured. The swab smeared with otic material was placed back into the original tube. All such samples were numbered. Two swabs were collected from each ear, one for bacteriological

investigation and other for fungal studies.

TABLE - IX.

Number of samples examined from buffaloes and dogs.

Samples collected from	Buffaloes		Dogs	
	No. of animals	No. of samples	No. of animals	No. of samples
(A) Infected ear				
1. Only one ear infected	2	2	10	10
2. Both ears infected	24	48	6	12
(B) Normal ear				
1. One ear normal	2	2	8	8
2. Both ears normal	9	18	6	12
Grand total.	37	70	30	42

(C) Bacteriological examination.

For the isolation of different organisms the swabs were inoculated on sheep blood agar and MacConkey's agar plate and was incubated aerobically overnight at 37°C. After incubation colonies were examined by magnifying glass and by transmitted light for their colonial characteristics. Different colonies were picked up and transferred to blood agar slants if the colonies were suspected for Streptococcus and Staphylococcus and nutrient agar slants if organisms were suspected to belong

to other genera. The slant cultures were further examined in details.

Test for purity of isolated strains :

The slant cultures were tested for purity after staining with the Gram's method. Morphology was noted for each one of them. Cultures of genus Streptococcus and Staphylococcus were grown in glucose broth whereas others were subcultured in nutrient broth. The pure cultures were then subjected to primary and secondary tests. In general, the methods given in the "Manual for identification of Medical Bacteria" by Cowan and Steel (1970) were followed throughout the study.

Primary tests :

1. Motility : - An eighteen hour broth culture incubated at 37°C was examined microscopically in a hanging drop preparation.

2. Catalase activity : - A loopful of the culture from the pure culture slant was taken on a clean slide and a drop of 3.0% H_2O_2 was added over it and examined for the production of gas bubbles which indicated a positive reaction or alternatively a few drops of 3.0% H_2O_2 were added to the growth on the nutrient agar slant and vigorous production of gas bubbles was taken as positive for the test.

3. Oxidase activity : - The test was done by dropping

oxidase reagent (1.0% aquous solution of tetramethyl-p-phenelene diamine) on the filter paper and rubbing the organism on the moist surface. Positive reaction was indicated by the development of dark purple colour on the paper within a few seconds.

4. Oxidation or fermentation of glucose : - Two tubes of Hugh and Leifson medium (Hugh and Leifson, 1953) were inoculated by stabbing with a straight wire. One of the tubes was layered with melted soft paraffin to a depth of about 1 cm. Both tubes were incubated at 37°C and examined daily upto 14 days. Yellow colour in both tubes indicated fermentation, yellow colour only in open tube indicated oxidation and no change in colour in both the tubes indicated negative for both the tests.

Secondary tests :

1. Aesculin hydrolysis : - Aesculin agar was prepared as per the recommendations of Cowan and Steel (1970) and it was inoculated with the pure culture. Plates were incubated at 37°C for seven days and examined daily during that period. Blackening of the medium around the colonies indicated a positive result.

2. Coagulase test : - 0.1 ml of 24 hours broth culture was added to 0.5 ml of diluted rabbit plasma (1 in 10) and incubated at 37°C for 1 to 6 hours. The tubes were observed for coagulase production at one, three and six hours intervals. Negative cultures were left at room temperature overnight and then re-examined as above.

3. Gelatin liquefaction : - Gelatin agar plates were inoculated with the culture and incubated at 37°C for 3 days. The surface of the incubated plates were flooded with 5 - 10 ml acid mercuric chloride solution. The positive results were indicated by a clear zone of gelatin hydrolysis.

4. Citrate utilization : - A slant of the Simmon's citrate agar was inoculated by making a single streak over the surface. The slants were incubated at 37°C for 7 days and were examined daily for growth and colour change. Appearance of blue colour indicated positive result.

5. Hydrogen sluphide production : - Tubes of the tripple sugar iron agar (T.S.I.) were inoculated by stabbing the butt and streaking the slope. Tubes were incubated at 37°C for 7 days and examined daily for blackening due to H_2S production. Alternatively, the organism was grown in nutrient broth and a lead acetate paper was inserted between the plug and tube. The tubes were incubated at 37°C for 7 days and examined daily for blackening of the paper during that period.

6. Indole production : - One ml oxylol was added to a 48 hours nutrient broth culture and shaken well. After that 0.5 ml of the Ehrlich's reagent was run down the side of the tube. The development of pink colour indicated the presence of indole.

7. Methyl red (M.R.) and Voges-Proskauer test : - Cultures were grown in glucose phosphate medium for 3 days and divided into two tubes. In one tube two drops of 0.04% methyl

red solution was added. In positive M.R. reaction, it became red whereas in negative case it remained yellow.

In the other tube 0.6 ml of 5.0% alpha-naphthol solution followed by 0.2 ml of 40.0% KoH solution was added. The tube was placed in sloping position. The positive reaction was indicated by appearance of brick red colour within 15 minutes.

8. Nitrate reduction : - The organisms were grown in 0.1% nitrate broth and incubated for 5 days. Presence of nitrites was tested by adding 1.0 ml of Nitrate reagent A (0.8% Sulphanilic acid in 5N acetic acid) followed by 1.0 ml of Nitrate reagent B (0.6% alpha-naphthylamine in 5N-acetic acid). Appearance of red colour indicated presence of nitrites.

In the negative tubes, zinc powder about 5 mg/ml was added to determine the presence of nitrate in the medium. Production of red colour indicated presence of nitrate in the medium, that is, nitrate was not reduced by the organism. Absence of red colour indicated the absence of nitrate in the medium, that is, the nitrate was reduced to nitrites and further to ammonia.

9. Pigment production for Pseudomonas : - King's agar A and B media were prepared as per the recommendations of King et al. (1954). The media were inoculated and incubated at 37°C for 1 to 4 days and examined for pigment production during that period.

10. Urease activity : - Slants of Christensen's urea agar were inoculated and incubated at 37°C for 5 days.Tubes

were examined first after four hours and then once daily for 5 days. Positive result was indicated by red colour of the medium.

11. Carbohydrate fermentation : - The peptone water media with following sugars were used to study acid or acid and gas production - glucose, lactose, maltose, sucrose, arabinose, mannitol and sorbitol.

(D) Mycological examination.

For the isolation of fungi, all the samples were inoculated separately in the tubes of the Sabouraud's dextrose agar with Chloromycetin (0.05 mg/ml). The tubes were incubated at room temperature (25-30°C), for four weeks and examined daily. No tube was discarded as negative until 4 weeks of incubation. Whenever there was contamination with bacteria or other fungi sub-culture was made on slant containing Streptomycin (0.05 mg/ml) to obtain pure culture. The separation of yeast contaminated with bacteria and fungi was made as described by Ajello et al. (1963).

Procedure for separating yeast mixture :

The yeast was inoculated in the tubes of the Sabouraud's dextrose broth and incubated at 37°C. After overnight incubation tubes were shaken and subcultured on blood agar and incubated at 37°C overnight. A single desired colony was picked

up from the plate and transferred to a Sabouraud's dextrose agar slant and incubated at 37°C overnight.

Procedure for separating bacterial contaminants :

Four tubes of the Sabouraud's dextrose broth with 1 drop, 2 drops, 3 drops and 4 drops of 1 N HCl respectively were inoculated with fungal growth. All the tubes were incubated overnight at 37°C. Subcultures were made from the above tubes to blood agar plates. Colonies were picked up from the plate which was free from bacterial contamination.

Identification of fungi :

Following studies were made for the identification of filamentous fungi.

The colonies of fungi were examined for their morphological characteristics such as, rate of growth, general topography (fluffy, heaped, flat, round, irregular and folded), their texture (glabrous, powdery, granular, valvety and cottony). The pigmentation on the surface and on the reverse side was also recorded.

Microscopical examination of colony and edge of the medium was done to have the preliminary idea for the type of fungus and their sporulation. A portion of the colony was placed on a slide in a drop of lactophenol cotton blue. The structure was teased apart and covered with a cover slip. The edge of the cover slip was sealed with nail polish to check evapora-

tion. The preparation was examined under 10X objective of light microscope.

Ridell's slide culture method was resorted to whenever necessary to study the detailed morphology (Ridell,1950).

Identification of yeasts :

Yeasts were stained with the Gram's method to study their morphology; however, their detailed studies could not be done as facilities were not available for "Sugar assimilation test".

Experimental production of otitis externa in buffalo calves, dogs and rabbits :

Experimental production of otitis externa was tried with Staph. aureus in buffalo calves and rabbits and with Pseudomonas aeruginosa in buffalo calves, dogs and rabbits.

Preparation of animal :

Apparently healthy buffalo calves, dogs and rabbits purchased locally were used. Animals were maintained on usual feeds and fodder. Swabs were taken from each ear before the start of the experiment and inoculated on blood agar and MacConkey's agar to know the normal micro-organisms of the ear. Blood agar and MacConkey's agar plates were incubated at 37°C overnight.

Only one ear canal was used for the experimental

study and the other ear was kept as control in each animal. The ear canals of animals were examined regularly for any inflammation, seddening, discharge during the period of observation. Ear swabs were taken at the interval of 6, 10 and 15 days for the bacteriological examination.

Preparation of bacterial suspension :

Organisms were subcultured from the pure stock culture on nutrient agar slants and incubated at 37°C for 48 hours to obtain good growth. A suspension of the culture was prepared by pouring 2 ml of sterile physiological saline solution in each culture tube and the growth was emulsified with the help of platinum loop. The whole content was taken out in a sterile tube with the help of pippete. The suspension was matched with different Brown's opacity tubes to judge the approximate number of organisms per ml.

Infection with Staphylococcus aureus :

Six buffalo calves of about one year age, and six rabbits were used for infection with Staph. aureus. One ml and 0.5 ml of saline suspension of Staph. aureus compared with the Brown's opacity tube No. 5 (15,000000 organisms/ml approximately) were poured in the left ear of three buffalo calves and three rabbits respectively. The ear was kept in upright position for two to three minutes and rubbed moderately and plugged with cotton.

In the other three buffalo calves and rabbits the infection was given in the same way except that a single line of scarification was made in the external ear canal with the help of a sterilized needle before instillation.

Infection with Pseudomonas aeruginosa :

In this case, three buffalo calves, three dogs and three rabbits were subjected to infection by instillation and other three buffalo calves and three rabbits by the scarification method. One ml of saline suspension compared with the Brown's opacity tube No. 5 was used in buffalo calves and dogs and 0.5 ml suspension was used in rabbits. Rest of the method was similar as adopted in the previous experiment.

Drug sensitivity trial :

The following antibiotic discs received from M/s. Pasteur Biological Laboratories, Umbergaon, District - Bulsar, Gujarat were used to study the sensitivity of various isolates to various antibiotics : Penicillin G, Oxy tetracyclines, Chlortetracyclines, Streptomycin, Kanamycin, Erythromycin, Polymyxin-B, Chloramphenicol and Neomycin.

Disc method as described by Cruickshank (1970) in the Medical Microbiology, Eleventh Ed. Published by "The English Language Book Society and Mls. Livingston Ltd. was used for the sensitivity test to antibiotics. Eighteen hour old broth culture of each isolate was spread over the nutrient

agar plate with the help of sterile swabs. Plates were allowed to dry in an inverted position in the incubator for 30 minutes. The plates were marked and numbered at four to five places according to antibiotics used. Antibiotic discs were placed on the surface of culture medium against their respective numbers marked on the opposite side of the plate. Plates were incubated at 37°C overnight and the zone of inhibition was measured the following day. The zone included the diameter of disc as well as sorrounding zone of inhibition.

The interpretation of results was made according to the interpretative table supplied by the manufacturer as presented in Table - X.

TABLE - X.

Interpretative table for antibiotics.

Antibiotic	Disc potency	Diameter (mm) of zone of inhibition		
		Resistant	Intermediate	Sensitive
AMPICILLIN -				
(a) Gram negative and enterococci	10 mcg	11 or less	12 - 13	14 or more
(b) Staphylococci and Penicillin-G sensitive micro-organisms.	10 mcg	20 or less	21 - 28	29 or more
PENICILLIN-G				
(a) Staphylococci	10 U	20 or less	21 - 28	29 or more
(b) Other micro-organisms	10 U	11 or less	12 - 21	22 or more
TETRACYCLINE	30 mcg	14 or less	15 - 18	19 or more
STREPTOMYCIN	10 mcg	8 or less	12 - 14	15 or more
KANAMYCIN	30 mcg	13 or less	14 - 17	18 or more
ERYTHROMYCIN	15 mcg	13 or less	14 - 17	18 or more
POLYMYXIN B	300 U	9 or less	9 - 11	12 or more
CHLORAMPHENICOL	30 mcg	12 or less	13 - 17	18 or more
NEOMYCIN	30 mcg	12 or less	13 - 16	17 or more

RESULTS

R E S U L T S

A total of 72 cases were investigated both in buffaloes and dogs. It is presented in Table - XI. Of the 26 buffaloes and 16 dogs which were suffering from otitis externa, 24 buffaloes and six dogs had bilateral involvement whereas two buffaloes and ten dogs had infection in only one ear.

TABLE - XI.

Frequency of involvement of ears in otitis externa.

No. of animals		Bilateral involvement	Unilateral involvement	Total no. of ears examined
Buffaloes	26	24	2	50
Dogs	16	6	10	22

The incidence according to the breed of buffaloes and dogs suffering from otitis externa is presented in Table - XII.

TABLE - XII.

Incidence of otitis externa according to the breeds of animals.

Buffaloes	- Murrah	- 7
	- Non-descript	- 19
Dogs	- Alsatian	- 7
	- Cocker spaniel	- 3
	- Pekingese	- 1
	- Dachshund	- 1
	- Non-descript	- 4

The incidence according to age and sex of animals suffering from otitis externa is presented in Table - XIII.

TABLE - XIII.

Incidence of otitis externa according to age and sex.

Species of animals	Age	No.of cases	No.according to sex	
			Male	Female
Buffaloes	Below 3 years	1	4	22
	3 years to 6 years	11		
	Above 6 years	14		
Dogs	Below 2 years	Nil	10	6
	2 years to 4 years	4		
	Above 4 years	12		

It can be seen from Table - XIII that most cases of otitis externa was recorded in animals above four years of age in dogs and above six years of age in buffaloes. The number of cases of otitis externa in male dogs was higher than that in females while in buffaloes the number of cases was higher in she-buffaloes.

Different kinds of micro-organisms isolated from the infected and normal ears of buffaloes are presented in Table - XIV and XV and Figures one and two respectively.

It is evident from the Table - XIV that though Staph. aureus, Staph. epidermidis, Str. faecalis, P. vulgaris

TABLE - XIV.

Micro-organisms isolated from infected and normal ears of buffaloes

Micro-organisms	Infected		Normal	
	No. of cases from which isolated*	Per - centage	No.of cases from which isolated*	Per- centage
Staphylococcus aureus	30	60	1	5
Staph. epidermidis	8	16	8	40
Streptococcus B-haemolyticus	2	4	Nil	
Str. faecalis	1	2	1	5
Micrococci	8	16	4	20
Aerobic spore bacilli	21	42	10	50
Pseudomonas aeruginosa	5	10	Nil	
Escherichia coli	3	6	Nil	
Citrobacter freundi	1	2	Nil	
Enterobacter aerogenes	2	4	Nil	
Proteus mirabilis	6	12	Nil	
Proteus vulgaris	1	2	1	5
Morexella sp.	1	2	Nil	
Chromobacterium lividum	1	2	Nil	

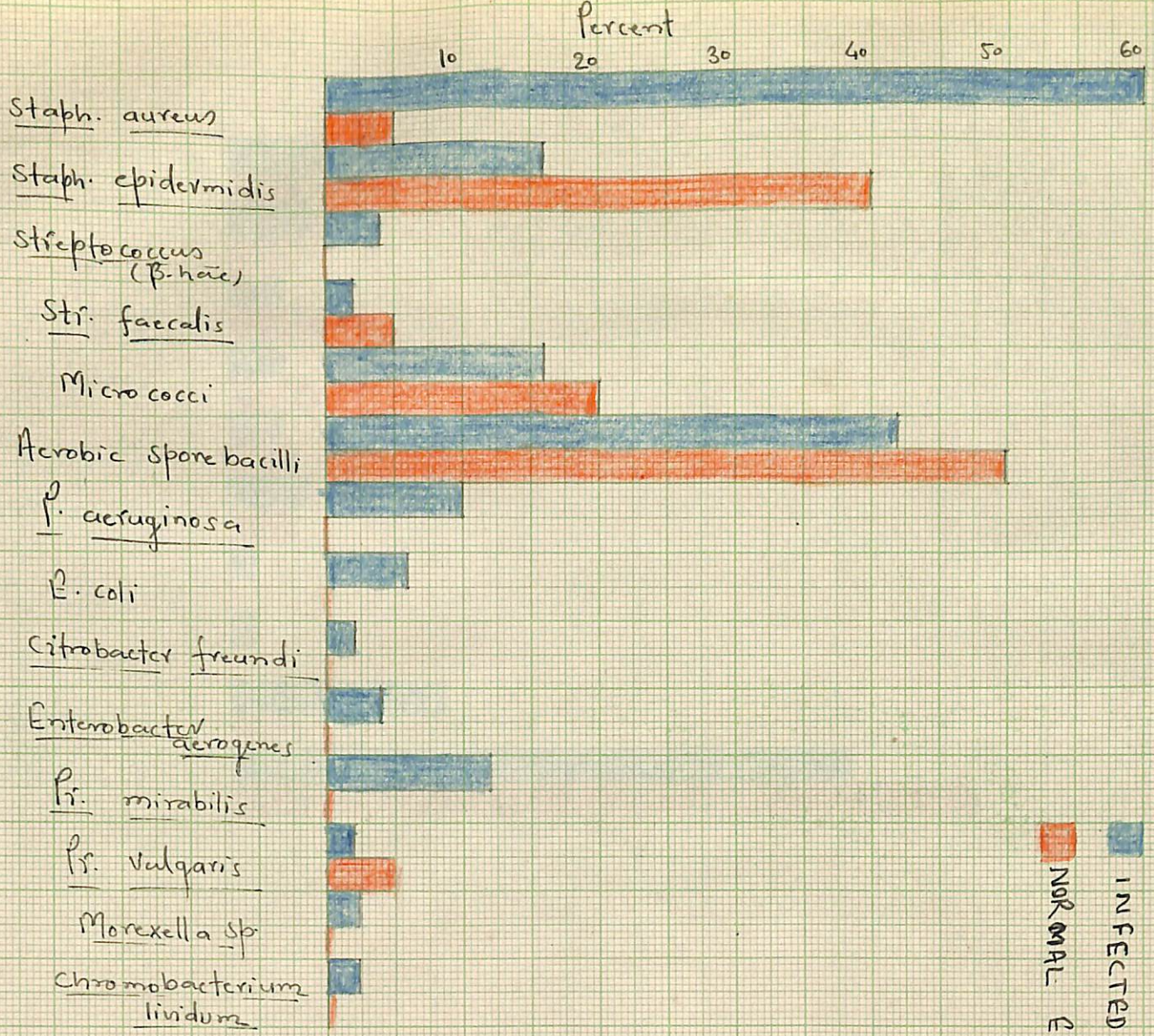
* A total of 50 infected and 20 normal ears were examined and percentage is calculated on this.

TABLE - XV.

Different fungi isolated from infected and normal ears of buffaloes

Species of fungi	Infected		Normal	
	No.of cases from which isolated	Per-centage	No.of cases from which isolated	Per-centage
Aspergillus niger	6	12	1	5
A. flavus	3	6	1	5
A. fumigatus	2	4	2	10
A. nidulans	2	4	2	10
Rhizopus sp.	2	4	1	5
Alternaria sp.	3	6	1	5
Penicillium sp.	1	2	Nil	
Fussarium sp.	1	2	Nil	
Yeasts	8	16	1	5
No growth	23	46	11	55

BACTERIOLOGICAL FINDINGS OF INFECTED AND NORMAL EARS OF BUFFALOES



Mycological Findings of Infected and Normal Ear of Buffaloes

were isolated from both infected and normal ears, in addition to micrococci and aerobic spore bacilli, the rate of isolation of Staph. aureus was quite higher in the former group. Str. β- haemolyticus, Pseudomonas aeruginosa, Escherichia coli, Citrobacter freundi, Enterobacter aerogenes, Proteus mirabilis, Morexella and Chromobacterium lividum were isolated only from infected ears. The rate of isolation of Staph. epidermidis, Str. faecalis, micrococci and aerobic spore bacilli was higher from normal ears.

Similarly, it is evident from Table - XV that Aspergillus niger and yeasts were present in high percentage in infected ears than normal ears. Fungi of the Penicillium sp. and Fussarium sp. were isolated only from infected ears.

The various bacteria and fungi isolated from infected and normal ears of dogs are presented in Tables - XVI and XVII and Figures 3 and 4 respectively.

It is evident from Table - XVI that only Staph. aureus, and Pseudomonas aeruginosa were isolated in significantly higher percentage from infected ears than the normal ears. Str. β-haemolyticus, E. coli, Citrobacter freundi and Proteus mirabilis were present only in infected ears.

Similarly, it can be seen from Table - XVII that Aspergillus niger and yeasts were present in significantly higher percentage in infected ears than the normal ears.

TABLE - XVI.

Different species of bacteria isolated from infected and normal ears of dogs

Micro-organisms	Infected		Normal	
	No. of cases from which isolated*	Per-centage	No. of cases from which isolated*	Per-centage
Staphylococcus aureus	8	36.36	1	5
Staph. epidermidis	2	9.09	11	55
Streptococcus β-haemolyticus	2	9.09	Nil	
Str. faecalis	1	4.54	1	5
Micrococci	3	13.63	2	10
Aerobic spore bacilli	8	36.36	12	60
Pseudomonas aeruginosa	6	27.27	1	5
Escherichia coli	2	9.09	Nil	
Citrobacter freundi	2	9.09	Nil	
Proteus mirabilis	2	9.09	Nil	
No growth	Nil		1	5

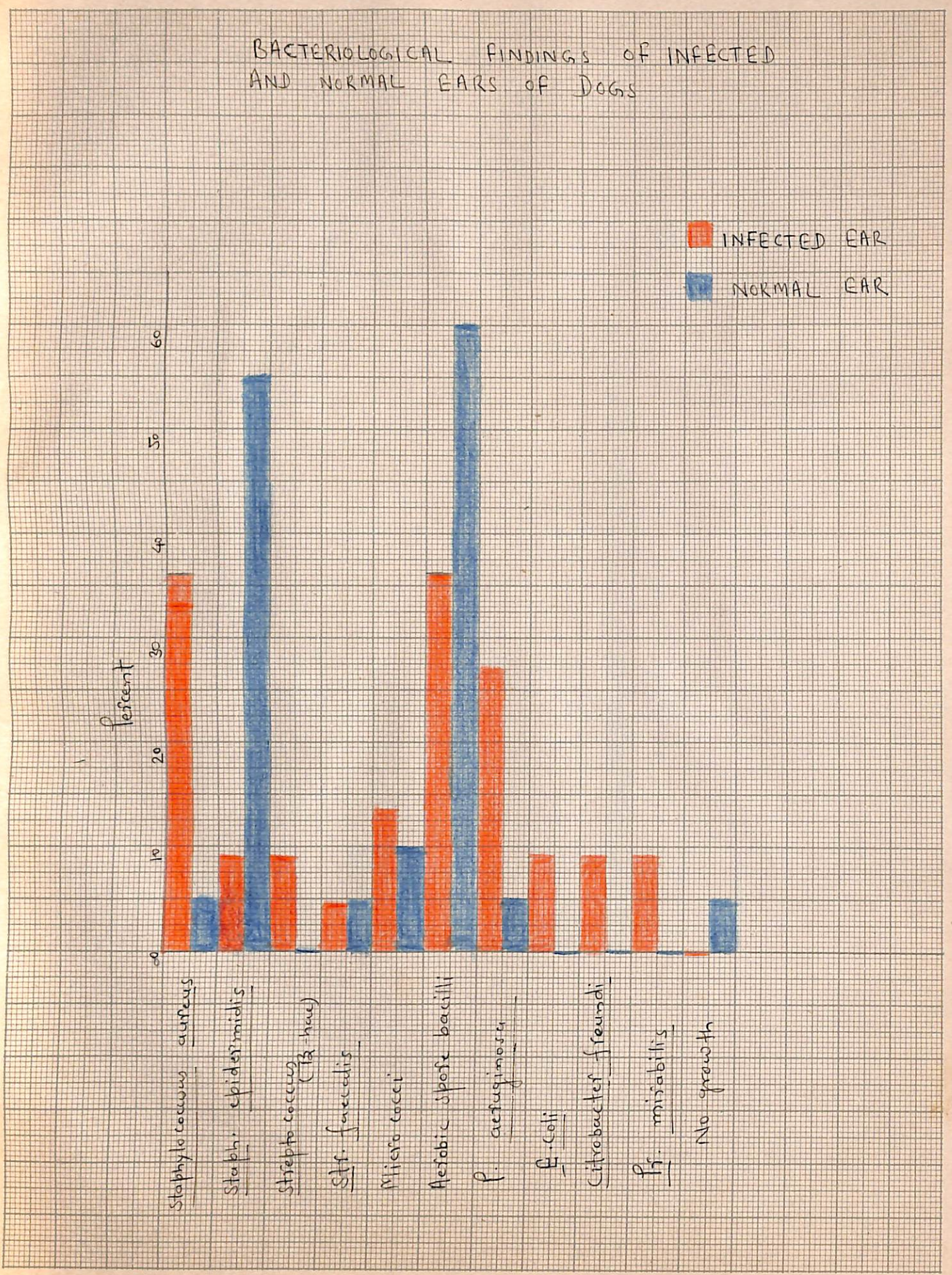
* A total of 22 infected and 20 normal ears were examined and percentage is calculated on this.

TABLE - XVII.

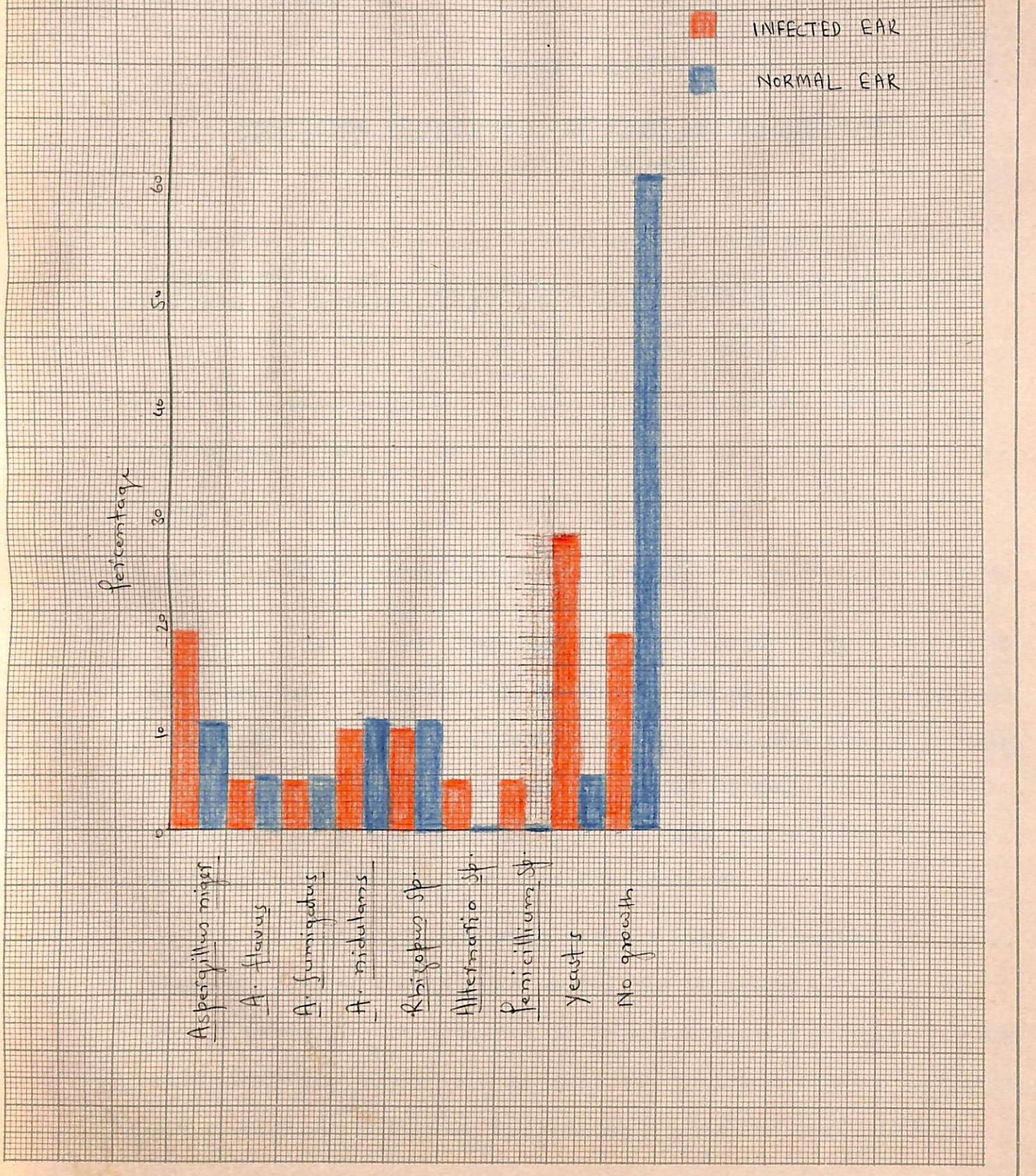
Different fungi isolated from infected and normal ears of dogs

Species of fungi	Infected		Normal	
	No.of cases from which isolated	Percen-tage	No.of cases from which isolated	Percen-tage.
Aspergillus niger	4	18.18	2	10
A. flavus	1	4.54	1	5
A. fumigatus	1	4.54	1	5
A. nidulans	2	9.09	2	10
Rhizopus sp.	2	9.09	2	10
Alternaria sp.	1	4.54	Nil	
Penicillium sp.	1	4.54	Nil	
Yeasts	6	27.27	1	5
No growth	4	18.18	12	60

BACTERIOLOGICAL FINDINGS OF INFECTED AND NORMAL EARS OF DOGS



Mycological Findings of Infected and Normal Ears of Dogs



The details of the isolation of bacteria and fungi from otitis externa in buffaloes and dogs are presented in Table - XVIII.

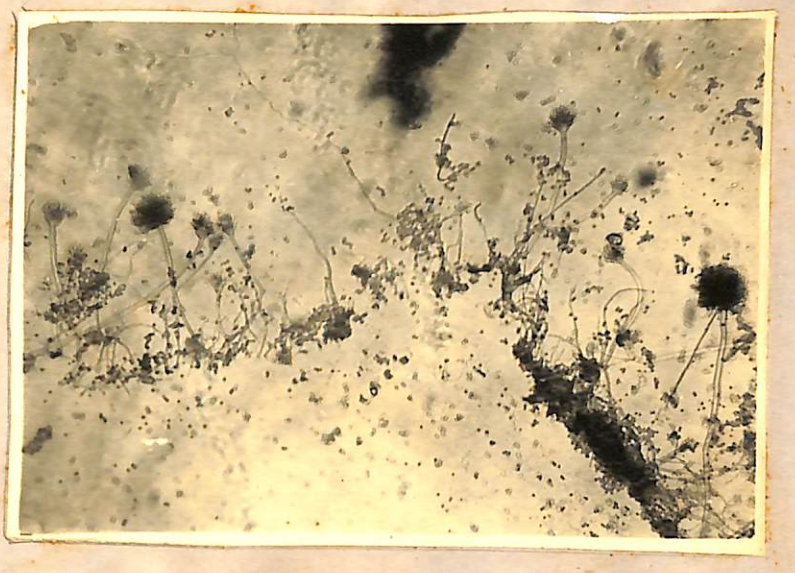
TABLE - XVIII.

Occurrence of bacteria and fungi in buffaloes and dogs with otitis externa

Species of animals	No. of infected ears	Bacteria			Fungi			Bacteria and fungi
		Only one sp.	More than one sp.	Total	Only one sp.	More than one sp.	Total	
Buffaloes	50	8	15	22	Nil	Nil		27
Dogs	22	3	7	10	1	Nil	1	11

It can be seen from the Table - XVIII that out of 50 infected ears of buffaloes, 22 showed the presence of only bacteria; 27 revealed the presence of both bacteria and fungi while none was infected by fungi only. Of the 22 ears which showed the presence of bacteria only, 8 had only one species of bacteria whereas 15 had more than one species.

Out of 22 infected ears of dogs, 10 showed the presence of bacteria only, one showed the presence of fungi only and 11 revealed the presence of both bacteria and fungi. Of the ten ears which showed the presence of bacteria only, 3 had only one species of bacteria, whereas more than one species were



Photograph showing slide culture of *Aspergillus niger*

were isolated from seven ears. One ear showed the presence of one species of fungus.

Table - XIX shows the comparison of isolation in buffaloes and dogs with both ears infected. It will be seen from this table that out of 24 such buffaloes and six such dogs, only one buffalo and one dog had the same type of infection in both ears, whereas 23 buffaloes and five dogs showed different types of isolation from both ears.

TABLE - XIX.

Comparison of microbial isolates in buffaloes and dogs with both ears infected

Isolations	No. of buffaloes	No. of dogs
Same isolations from both ears		
Bacteria	1	Nil
Fungi	Nil	Nil
Bacteria + fungi	Nil	1
Different isolations from both ears		
Only bacteria from both	2	2
Bacteria + fungi from both	6	1
Bacteria + fungi from one ear and only bacteria from other ear		
(a) Same bacteria	1	Nil
(b) Different bacteria	14	1
Bacteria and fungi from one ear and only fungi from the other ear.	Nil	1

Pathogenecity trial

Experiment No. I - infection with Staphylococcus aureus.

Bacteriological findings of the experimental buffalo calves and rabbits before and after infection are given in Table - XX.

After 6 days of inoculation symptoms of pain i.e. shaking of head was noticed in buffalo calves No. 1, 4 and 6. Swabs from ears of these three animals were positive for Staph. aureus. Symptoms of pain was noticed in rabbits No. 1, 4 and 5 during examination of ear. Slight redness was noticed in the ear canal of No. 4 and 5. Culture of the ear swab yielded profuse growth of Staph. aureus in No. 1, 4 and 5. On 10th day of examination brown-yellowish pus was noticed in the ear of buffalo calves No. 1, 4 and 6 and in rabbits No. 1, 4 and 5. Swab culture was positive for Staph. aureus. Varying degree of inflammation was noticed in these buffalo calves and rabbits.

On 15th day of examination, the pus became more sticky but the picture was more or less similar, as seen on the 10th day.

Experiment No. II - Experiment with Pseudomonas aeruginosa :

Results of culture from swabs taken before and after inoculation are presented in Table - XXI.

TABLE - XX.

Result of the experimental infection of otitis externa in buffaloes and rabbits with *Staph. aureus*

Species of animals	Number of animals					
	Infected by instillation			Infected by scarification		
	1	2	3	4	5	6
(a) Buffalo calves						
Culture before giving infection.	Aerobic spore bacilli	*Staph. epidermidis*	*Staph. epidermidis* + Micrococci	Aerobic spore bacilli + *Staph. epidermidis*	Aerobic spore bacilli + *Staph. epidermidis*	Aerobic spore bacilli
Culture after infection.	*Staph. aureus*	*Staph. epidermidis*	*Staph. epidermidis*	*Staph. aureus*	*Staph. epidermidis*	*Staph. aureus*
(b) Rabbits						
Culture before giving infection.	*Staph. epidermidis*	*Staph. epidermidis* + aerobic spore bacilli	Aerobic spore bacilli	Micrococci	*Staph. epidermidis*	*Staph. epidermidis* + aerobic spore bacilli
Culture after infection.	*Staph. aureus*	*Staph. epidermidis*	No growth	Profuse growth of *Staph. aureus*	Profuse growth of *Staph. aureus*	*Staph. epidermidis*

TABLE - XXI.

Result of the experimental infection of otitis externa in buffaloes, dogs and rabbits with Pseudomonas aeruginosa

Species of animals	Number of animals					
	Infected by instillation			Infected by scarification		
	1	2	3	4	5	6
(a) Buffalo calves						
Culture before giving infection.	Aerobic spore bacilli + Micrococci	Staph. epidermidis	Staph. epidermidis	Staph. epidermidis	Aerobic spore bacilli + Micrococci	Staph. epidermidis
Culture after infection.	P. aeruginosa	P. aeruginosa	Scanty growth of P. aeruginosa	P. aeruginosa	P. aeruginosa	P. aeruginosa
(b) Rabbits						
Culture before giving infection.	Micrococci	Staph. epidermidis	Micrococci	Aerobic spore bacilli	Staph. epidermidis	Staph. epidermidis
Culture after infection.	No growth	Staph. epidermidis	No growth	Aerobic spore bacilli	Scanty growth of P. aeruginosa	P. aeruginosa
(c) Dogs						
Culture before giving infection.	Staph. epidermidis	Staph. epidermidis +Aerobic spore bacilli	Staph. epidermidis	NOT DONE		
Culture after infection.	Staph. epidermidis	Staph. epidermidis	Staph. epidermidis			

(a) Buffalo calves :

After the 6th day of examination, constant shaking of head was observed in all the animals. Swab culture of all the animals except No. 3 yielded profuse growth of P.aeruginosa. On the 10th day of examination, reddish-brown pus was noticed in all the animals except No. 3. Intense inflammation was noticed in buffalo calves No. 5 and 6. Slight redness was noticed in buffalo calves No. 1, 2 and 4. Heavy growth was observed on swab culture of buffalo calves No. 1, 2, 4, 5 and 6. Scanty growth was observed in buffalo calf No. 3. On 15th day of observation, the picture was more or less same as on 10th day.

(b) Rabbits :

No inflammation was observed in rabbits except rabbit No. 6, which yielded profuse growth of Pseudomonas aeruginosa on the 6th and 10th day of examination. Scanty growth of Pseudomonas aeruginosa was observed in rabbit No. 5 on the 6th day of examination. Reddish-brown pus was noticed only in rabbit No. 6 on the 10th day of examination.

(c) Dogs :

There was no evidence of inflammation in any of the three dogs during 15 days' observation. There was no growth of Pseudomonas aeruginosa in any swab cultures which were made at different intervals.

Results of the sensitivity test :

Altogether 48 isolates were subjected to sensitivity tests against 10 antibiotics. The measurement of the zone of inhibition was the yard stick for evaluating the sensitivity of a particular strain to that particular antimicrobial drug.

Interpretative table supplied by the manufacturer was strictly followed in grouping all the isolates into three degree of sensitiveness, that is, sensitive, intermediate and resistant. The result of drug resistance is given in Table - XXIII.

TABLE - XXIII.

A general pattern of drug resistance against different antibiotics

Antibiotics	Sensitive	Intermediate	Resistant
Penicillin G	14*	14	20
Oxytetracycline	13	12	23
Chlor-tetracycline	14	13	21
Streptomycin	41	1	6
Amphicillin	13	14	21
Kanamycin	35	5	8
Erythromycin	26	5	17
Polymyxin B	4	3	41
Chloramphenicol	30	15	3
Neomycin	40	4	4

* No. indicates the total no. of strains sensitive, intermediate or resistant to that particular drug.

Photograph showing the sensitivity of E. coli strain with the following antibiotics:

1. Chloramphenicol
2. Kanamycin
3. Erythromycin
4. Chlortetracycline
5. Ampicillin
6. Oxytetracycline
7. Penicillin G
8. Neomycin
9. Streptomycin

It will be seen from Table - XXII that in general four antibiotics were effective against most of the isolates. They are chloramphenicol, neomycin, streptomycin and kanamycin. They were effective against 45, 44, 42 and 40 isolates respectively. Erythromycin, penicillin G, chlor-tetracycline, amphicillin and oxytetracycline were effective against 31, 28, 27, 27 and 25 isolates respectively. Polymyxin B was the least effective antimicrobial drug. It was effective only against 7 isolates.

Table - XXIV gives the sensitivity of different micro-organisms to different antibiotics. It will be seen from it that most strains of staphylococci were sensitive and intermediate to all antibiotics except polymyxin-B. Micrococci were sensitive to chloramphenicol, neomycin, kanamycin, penicillin G, streptomycin, erythromycin and oxytetracycline. Aerobic spore bacilli were mostly sensitive to chloramphenicol, streptomycin, erythromycin, kanamycin and neomycin. They were resistant to other five drugs. All the Pseudomonas were sensitive only to streptomycin except one which was in addition sensitive to chlortetracycline and neomycin. Strains of E. coli were slight to intermediately sensitive to kanamycin, chloramphenicol, chlortetracycline and neomycin. One strain was in addition sensitive to streptomycin. Proteus strains were sensitive to chloramphenicol, tetracyclines, neomycin and streptomycin.Three strains were in addition sensitive to penicillin G, amphicillin, and kanamycin and one to erythromycin. Organisms of the genus

TABLE - XXIII.

Sensitivity of different micro-organisms to different antibiotics

Micro-organisms	Sensitivity grade	Penicillin G	Oxytetracyclines	Chlortetracyclines	Erythromycin	Streptomycin	Amphicillin	Kanamycin	Polymyxin B	Chloramphenicol	Neomycin
Staphylococcus	S	6	7	3	7	7	1	8	-	8	7
	I	3	2	5	2	-	7	-	1	1	2
	R	-	-	1	-	2	1	1	8	-	-
Micrococcus	S	4	3	1	3	4	-	5	-	5	5
	I	-	2	2	1	-	4	-	1	-	-
	R	1	-	2	1	1	1	-	4	-	-
Aerobic spore bacilli	S	4	3	2	9	10	-	9	-	10	9
	I	1	3	2	1	-	4	1	-	-	1
	R	5	7	6	-	-	6	-	10	-	-
Pseudomonas	S	-	-	-	-	5	-	-	-	1	-
	I	-	-	-	2	-	-	-	-	1	2
	R	5	5	5	3	-	5	5	5	3	3
E. coli	S	-	-	-	-	1	-	2	-	2	-
	I	-	1	2	-	-	-	-	-	-	2

Citrobacter were sensitive only to amphicillin, kanamycin, chloramphenicol and neomycin. One of the strains was in addition sensitive to streptomycin while the other was sensitive to tetracyclines. Enterobacter strains were sensitive to all antibiotics except oxytetracycline, erythromycin, penicillin G and polymyxin-B. The single Morexella strain was sensitive to erythromycin, streptomycin, chloramphenicol and neomycin, while the Chromobacterium was sensitive to streptomycin, kanamycin and neomycin.

DISCUSSION

D I S C U S S I O N

Otitis externa is regarded as the inflammatory condition of the external auditary canal from its visible orifice to the tympanum. This is the most common condition in small animals such as, dogs and cats, but frequently it is also encountered in other animals like buffaloes and cattle.

In India, however very few attempts have been made to study the otitis externa in dogs and almost no serious attempt has been made to study the disease in buffaloes.

Several etiological and predisposing factors have been listed for the pathogenesis of otitis externa. These include bacteria, fungi, parasites, skin diseases, tumours, anatomical conformation of the ear, moisture, foreign bodies and the accumulation of dirt, wax and shed epithelium according to Riser (1949).

Some breeds of dogs are more susceptible to otitis externa. Breeds like cockerspaniel and poddle with their pendent and floppy ears and peculier anatomy of the ear canal are more prone to this disease. But in the present study Alsatian dogs with erect ear were found to suffer from this disease more than any other breed. This result does not confirm the observation of other workers like Joshua (1958) and Fraser (1961). But Mathew et al. (1970) who have also found the higher incidence of otitis externa in Alsatian breed are

of the opinion that the possibility of erect ears in harbouring smoke, dust and other extraneus particles is more than the floppy ears. This in turn increases the incidence of otitis externa in breeds with erect ear.

Although more cases of otitis externa were found in Murrah buffaloes, breed cannot be supposed to be a predisposing factor in otitis externa of buffaloes. There is apparently no difference in the size and anatomy of ear canal in different breeds. The lone factor which seems to be responsible in production of otitis externa in buffaloes, is the presence of moisture in the ears from dirty water as buffaloes are habituated for wallowing in ponds and ditches. In addition, cases were observed mostly in flooded areas. Presence of moisture in the ear favours the multiplication of micro-organisms.

Adult animals both in dogs and buffaloes accounted for 75 and 50 per cent of cases respectively. It may be due to increased sebaceous secretion and accumulation of wax in adult animals.

More cases of otitis externa were encountered in male dogs and in she buffaloes. However, the figure is not large enough to draw any conclusion regarding prevalence of otitis externa in different sexes.

It is generally seen that several species of micro-organisms (bacteria and fungi) are commonly found in affected as well as normal ears. But some micro-organisms are isolated

more frequently from the affected ears than the normal ears. In the present study Staph. aureus, Staph. epidermidis, Str. faecalis, micrococci and aerobic spore bacilli were isolated from normal as well as affected ears in both buffaloes and dogs. Pseudomonas aeruginosa was isolated from normal and affected ears of dogs. But the rate of isolation of only Staph. aureus was significantly more from affected ears (60.0 and 36.36% respectively in buffaloes and dogs) as compared to normal ears (16.0 and 9.09%) in both the animals. In dogs however P. aeruginosa was isolated from 27.06% of the affected ears. Other organisms were isolated either more frequently from normal ears in comparison to infected ears or were present in the same ratio in both ears. So only Staph. aureus can be regarded as pathogenic for both buffaloes and dogs and P. aeruginosa for dogs only.

Some bacteria such as, Streptococcus β-haemolyticus, E. coli, Citrobacter freundi and Proteus mirabilis were isolated only from infected ears of buffaloes and dogs. The percentage of their isolation in buffaloes was 4.0, 6.0, 2.0 and 12.0 respectively whereas in dogs it was 9.09 for all. Besides these, P. aeruginosa, Enterobacter aerogenes, Chromobacterium lividum and Moraxella were isolated only from infected ears of buffaloes. They were isolated in the percentage of 10.0, 4.0, 2.0 and 2.0 respectively. Since these organisms were not found in normal ears, their presence in otitis externa is of significance as they could be the etiological importance.

Micro-organisms like Staph. epidermidis, Str. faecalis, micrococci and aerobic spore bacilli were isolated from both species and Proteus vulgaris was isolated from buffaloes either with the same frequency from both ears or with higher rate from normal ears. So these micro-organisms can be regarded as normal flora.

It will be seen from the mycological findings of infected and normal ears of buffaloes and dogs (Table XV and XVII) that a large number of fungi were isolated from infected as well as from normal ears. Aspergillus niger and yeasts were isolated more frequently from infected ears than from normal ears. The percentage of these two fungi were 12.0 and 16.0 respectively in buffaloes and 18.18 and 27.27 respectively in dogs. Other fungi such as, Aspergillus flavus, Aspergillus fumigatus, Rhizopus sp. were found in both species of animals and Alternaria sp. were found to be present in buffaloes either in the same frequency in both types of ears or occurred more frequently in normal ears. Some of the other fungi such as, Penicillium sp., Fussarium sp. (in buffaloes) and Alternaria sp. (in dogs), were isolated from infected ears only but their rate of isolation was very less and insignificant. So on the basis of present study Aspergillus niger and yeasts can be regarded to be of pathogenic importance in otitis externa.

In most of the cases more than two types of organisms were observed. Even in animals which had bilateral infection, different types of organisms were isolated. Out of

the 24 buffaloes and six dogs having both ears infected, only one buffalo and one dog had the same etiological agent (Table XIX) in both ears. This points out that the type of infection in otitis externa is exogenous. It also denotes the complicated nature of the condition.

Staphylococcus aureus :- McBride (1953) was of the opinion that Micrococcus pyogenes var. aureus and albus were the most common cause of otitis externa and were isolated from 26.5% of cases. Gustafson (1955) observed that staphylococci were similar in both infected and normal ears. But 97.0% of staphylococci from infected ears produced toxin as compared with only 57.0% of staphylococci from normal ears. Fraser (1961a, 1961b) also reported a high incidence of Staphylococcus in otitis externa. Grono isolated staphylococci (coagulase positive) from 30.9% of infected ears and 47.6% from healthy ears. The incidence of Staphylococcus aureus in the present study is compared with other workers (Table XXIV). Staphylococcus aureus was observed to be the commonest organism present in otitis externa in both buffaloes and dogs. It was isolated in the percentage of 60.0 and 36.36 respectively from buffaloes and dogs.

Pseudomonas sp. :- Farrag and Mahmaud (1953) found a high incidence of Pseudomonas aeruginosa in ottorrhoea of dogs. Jones (1955) isolated Pseudomonas aeruginosa from 17 cases of otitis externa out of 128 infected ears. Gustafson (1955) regarded Pseudomonas aeruginosa as a secondary invador

as it occurred in otitis externa of long duration.

During the present study Pseudomonas aeruginosa was isolated from 10.0% and 27.26% respectively from infected ears of buffaloes and dogs. In buffaloes Pseudomonas aeruginosa was mostly found associated with Staphylococcus aureus. So it may be concluded that Pseudomonas aeruginosa is secondary invader in otitis externa of buffaloes. But in dogs it was found either alone or mostly associated with aerobic spore bacilli and micrococci, it may be called primary pathogen in these cases. This supports the view of Grono and Frost (1969) who reported a high incidence of Pseudomonas sp. (34.6) in infected ears as compared with normal ears (2.4%).

Proteus :- Proteus mirabilis and P. vulgaris were isolated from both buffaloes and dogs' infected ears in the ratio of 14.0% and 9.09% respectively as compared with 2.0% of normal ears of buffaloes whereas none from dogs' ears. Gustafson (1954) isolated Proteus sp. from 14.0% of the infected ears of dogs only. He concluded that Proteus almost occurs in otitis of long duration and regarded them as secondary invadors. Grono and Frost (1969) isolated Proteus sp. from 20.8% and 1.6% of the infected and normal ears of dogs respectively. In the present study in buffaloes Proteus sp. were found associated with Staphylococcus aureus (4.0%), Micrococcus (4.0%), Staphylococcus epidermidis, Chromobacterium and Enterobacter (2.0%). Thus in majority of these cases Proteus may be regarded as primary pathogen. In dogs organisms of the genus

Proteus were found without any association with other organisms. So in this case also it may be regarded as primary pathogen.

Streptococci have been found associated with otitis extern. In the present study β-haemolytic streptococci were isolated in the percentage of 4.0 and 0.09 respectively from the infected ears of buffaloes and dogs. Normal ears did not show β-haemolytic streptococci. Gustafson (1955) isolated streptococci from 56 cases out of 201 cases of otitis externa of dogs. As all the streptococci occurred in combination with other micro-organisms it was given a secondary importance in the etiology of otitis externa. Grono and Frost (1969) isolated β-haemolytic streptococci from 7.4% cases of otitis externa in dogs. In the present study β- haemolytic streptococci were found to be associated with other micro-organisms in the infected ears of both buffaloes and dogs. So streptococci may be regarded as the micro-organism of secondary importance in causation of otitis externa.

Coliform organisms such as, E. coli, Citrobacter and Enterobacter usually occur in nature and may be present in exudative ears where bacteria multiply freely. In the present study, they were isolated in 12.0% and 18.18% respectively of infected ears of buffaloes and dogs. Normal ears were free from coliform organisms. They can be regarded as secondary invaders as they were always found to be associated with other micro-organisms.

Some organisms such as, Chromobacterium, and Morexella were isolated from infected ears in ratio of about 2.0 per cent which is very insignificant and may be regarded as rare organism in otitis externa.

Other micro-organisms such as, Staphylococcus epidermidis, Streptococcus faecalis, micrococci and aerobic spore bacilli, which were isolated either in the same percentage from infected and normal ears or were present more in normal ears can be best regarded as normal flora of external ear canal.

A comparative figure of these organisms which were associated with otitis externa in different countries is presented in Table - XXIV.

In the present study Aspergillus niger was isolated respectively from 12.0% and 18.18% of infected ears of buffaloes and dogs. It is interesting to note that Aspergillus niger was found to be always associated with other pathogenic micro-organisms such as, Staphylococcus aureus. This observation supports the view of Philips (1963) who considers that fungal infection of the ear uncomplicated with pyogenic infection is rare in dogs. Ainsworth (1954) is of the opinion that saprophytic moulds (Aspergillus fumigatus) may act as commensals, but may become virulent and lethal pathogens in certain circumstances.

Other fungi, such as, Aspergillus fumigetus, Aspergillus flavus, Aspergillus-nidulans. Rhizopus sp.,

TABLE - XXIV.

Comparative bacteriological findings of otitis externa in Boston, Stockholm, Edinburg, Brisbane, Bombay, New Delhi and Bihar (present study).

Micro-organisms	Bostone 128 dogs (McBride, 1953).	Stockholm 354 dogs (Gustafson, 1954).	Edinburg 451 dogs (Fraser, 1961a).	Brisbane 716 dogs (Grono & Frost, 1969)	Bombay 30 dogs (Mathew et al., 1970).	Delhi 38 dogs (Sinha, 1970).	Bihar	
							50 ears of buffaloes	22 ears of dogs
Staphylococcus aureus (Coagulase +ve)	26.50*	50.00	71.90	30.90	30.76	52.00	60.00	36.36
Streptococcus β - haemolytic	21.80	12.20	22.50	7.40	17.30	8.00	4.00	9.09
Pseudomonas aeruginosa	13.30	11.00	17.00	34.60	21.15	22.00	10.00	27.06
Proteus sp.	22.70	14.00	21.50	20.80	3.88	10.00	14.00	9.09

* Percentage is calculated on the number of cases.

Alternalia sp., Penicillium sp. and Fussarium sp. which were either present in insignificant frequency in infected ears or were isolated in the same frequency from the infected and normal ears could be regarded as normal flora of the ear. These fungi are ubiquitous in nature and can get enterence in the external ear canal and can settle down there.

Yeast may be of greater importance in otitis externa. Wanger et al. (1968) reported that otitis externa in dogs could be caused by Cryptococcus neoformans. Fraser(1961) observed that prolonged antibiotic therapy may result into mycotic-otitis with Candida sp. Smith (1968) reported a high incidence of Pityrosporum canis in otitis externa of dogs. In the present study yeasts were isolated from 16.0% and 27.27% of the infected ears of buffaloes and dogs respectively as compared with 5.0% from normal ears of both species of animals. In most of these cases yeasts were found to be associated with Staphylococcus aureus. In one case of buffalo it was found to be associated with Proteus and in another case it was associated with Micrococcus. It is difficult to discern whether staphylococci or yeasts were the true pathogens. In one case of dog it was not found to be associated with other organisms. But the number is so small that no conclusive opinion could be formed except that yeasts might be pathogens.

Infection with Staphylococcus aureus in buffalo calves and rabbits showed similar pattern of the disease with the instillation and scarification method. In the instillation

method one buffalo calf out of three and one rabbit out of three exhibited the characteristic symptoms of otitis externa and yielded Staph. aureus in pure culture whereas in scarification method two buffalo calves and two rabbits out of three in each case showed characteristic symptoms and pure culture of Staph. aureus was obtained from these lesions. This result shows that Staph. aureus has little capacity to invade the intact skin of buffalo calves and rabbits but they can infect to a greater extent when a little trauma is inflicted. Gustafson (1955) produced otitis externa in dogs with Staph.albus (toxin producing strain). He concluded that non-toxin producing strains may not be dangerous but strains that produce toxin must be considered pathogenic.

In experiment No. II six buffalo calves and six rabbits were subjected to experimental infection with Pseudomonas aeruginosa. Besides, three dogs were also infected with this organism by instillation method.

All the three buffalo calves of the scarification group and two buffalo calves of the instillation group showed characteristic symptoms of otitis externa and Pseudomonas aeruginosa was isolated in pure culture from them. The sixth buffalo calf also yielded a scanty growth of Pseudomonas aeruginosa although the symptoms of otitis externa was not observed by naked eye.

The symptoms of otitis externa were not manifested either in the rabbits or dogs which were infected by the instillation method. Only one rabbit in which scarification was done showed the symptoms of otitis externa and Pseudomonas

aeruginosa in pure culture was obtained from the lesion. Scanty growth of the organism was obtained in another rabbit infected by the same method. It is clear from the experiment that Pseudomonas aeruginosa is capable of invading intact as well as traumatized skin of the external ear canal of buffaloes although the role of other factors such as, moisture cannot be ruled out. Its presence in the otitis externa of buffaloes may be regarded as a cause of the disease. However, its ability to infect healthy ears of dogs and rabbits alone is very doubtful. But it has the capacity to produce the disease in rabbits if the skin is scarified. Its presence in otitis externa of dogs may be regarded as of secondary importance. Gustafson (1955), and Senturia and Carr (1958) failed to produce otitis externa in the intact ear canal of dogs and rabbits with Pseudomonas aeruginosa. While Farrag and Mahmoud (1953) succeeded in producing otitis externa in dogs with heavy saline suspension of Pseudomonas aeruginosa.

As etiological agents in otitis externa are different even in two ears of the same animal it becomes essential to study the sensitivity pattern of each isolate for the proper treatment of such cases. Mathew et al. (1970) found that Pseudomonas was resistent to furracin, terramycin and otoryl. Most of other organisms were sensitive to furracin. Dey et al. (1972) found tetracyclines, streptomycin and chloramphenicol as most effective antibiotics to most of the organisms isolated from cases of otitis externa of dogs.

In the present study it was observed that chloramphenicol, neomycin, streptomycin and kanamycin were most effective antimicrobial agents. They were effective to inhibit the growth of 45, 44, 42 and 40 strains respectively out of 48 subjected to the test. Conclusions can be drawn that these antibiotics are the drug of choice for the treatment of otitis externa. Some other antibiotics such as, penicillin G, tetracyclins, and amphicillin may also be used with satisfactory results. It was also observed that organisms of the genus Pseudomonas were the most resistant organisms being sensitive only to streptomycin.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSIONS

1. The microbiological study of 50 and 22 infected ears of buffaloes (*Bos bubalis*) and dogs (*Canis familiaris*) respectively along with 20 normal ears of both the species was undertaken with the object of isolating and identifying the causative agents.

2. The highest incidence of otitis externa was found in the Alsatian breed among dogs. Breedwise incidence was not recorded in buffaloes although more cases (26.5%) were observed in Murrah buffaloes among the cases studied.

3. Most of the cases of otitis externa were observed in adult animals. No conclusion was drawn regarding the incidence according to sex most probably due to small number of cases of different sexes observed.

4. It was found that *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Streptococcus* (β - haemolytic) and coliform bacilli were present in higher proportion in the infected ears as compared with normal ears.

5. Similarly, only *Aspergillus niger* out of different fungi isolated was present in higher proportion in the infected ears as compared with normal ears. In addition, yeast cells were also isolated in higher percentage from the infected ears.

6. Of the 26 buffaloes and 16 dogs investigated, 24 buffaloes and six dogs had bilateral infection. Only one buffalo and one dog had the same etiological agents in both the ears.

7. Twentytwo out of 50 ears of buffaloes and 10 out of 22 ears of dogs revealed the presence of bacterial organisms whereas 27 cases of buffaloes and eleven cases of dogs were due to both bacteria and fungi and in one case of dog only yeast cells were isolated.

8. Staphylococcus aureus was isolated from 14.0% of ears infected with only one type of organisms and Pseudomonas aeruginosa was isolated from 2.0% of such cases. In the remaining cases, Staph. aureus, Proteus mirabilis, Streptococcus (β- haemolytic), Morexella, E. coli, Staph. epidermidis and Str. faecalis were found to be associated with other bacteria giving the percentage of 12.0, 6.0, 2.0, 2.0, 2.0, 2.0 and 2.0 respectively.

9. Similarly, out of 10 ears of dogs which showed the presence of only bacteria, only one type of bacteria, that is, Proteus mirabilis and Pseudomonas aeruginosa were isolated from three ears giving the per cent of 9.09 and 4.54 respectively. In the remaining seven ears, Pseudomonas aeruginosa, Staph. aureus, Str. β - haemolyticus and micrococci were found to be associated with other organisms in the percentage of 6.0, 4.0, 2.0 and 2.0 respectively.

10. Only one ear of a dog was infected with a yeast.

11. Eighteen ears out of a total of 27 ears in buffaloes from which both bacteria and fungi were isolated, Staph. aureus was associated with Aspergillus niger, yeasts, Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Rhizopus sp., Penicillium sp. and Fussarium sp. giving the percentage of 10.0, 8.0, 6.0, 4.0, 2.0, 2.0, 2.0 and 2.0 respectively. In the remaining nine ears yeasts were associated with Staph. epidermidis, micrococci and Proteus mirabilis in three ears, Alternaria sp. with micrococci, Staph.epidermidis and aerobic spore bacilli in three cases respectively, Pseudomonas aeruginosa with Rhizopus sp. and Aspergillus nidulus in two ears and Streptococcus β- haemolytic was associated with Aspergillus niger in one ear.

12. Similarly, eleven ears of dogs which showed the presence of both bacteria and fungi, Staph. aureus was associated with yeasts, Aspergillus niger, Aspergillus fumigatus, Penicillium, Aspergillus nidulans, Rhizopus and Alternaria in seven ears. In the remaining four ears, Streptococcus β- haemolytic was associated with Aspergillus niger and Aspergillus fumigatus in one ear, Pseudomonas aeruginosa with Aspergillus niger and Rhizopus in one ear, Citrobacter with yeast in one ear, and aerobic spore bacilli with Aspergillus flavus and yeast in one ear.

13. Experimental infection with Staph. aureus in buffalo calves and rabbits showed typical signs and symptoms of otitis externa both by the instillation and scarification methods in the percentage of 33.3 and 66.6 respectively.

14. Infection with Pseudomonas aeruginosa in buffalo calves showed typical signs of otitis externa in 66.6% of animals by instillation method and 100.0% of animals by scarification method.

No signs and symptoms of otitis externa were produced in three rabbits and three dogs infected by the instillation method; however, only one rabbit (33.3%) could be infected by the scarification method.

15. It is concluded that Staph. aureus has little ability to invade the healthy skin of buffalo calves and rabbits but it can infect to a greater extent when some trauma is produced. Pseudomonas aeruginosa is capable of invading healthy as well as traumatized ear canal of buffalo calves. However, its ability to infect the healthy ear canal of dogs and rabbits is very doubtful.

16. Sensitivity tests of the different isolates against different antibiotics in vitro revealed that only four antibiotics, viz., chloramphenicol, neomycin, streptomycin and kanamycin are effective against most of the isolated organisms.

REFERENCES

R E F E R E N C E S

Ainsworth, G.C. (1954). Vet. Rec., 66: 844.

Ajello, L., Georg, L.K., Kaplan, W. and Kauffman, L. (1963). Laboratory manual for Medical Mycology Pub. Hlth. Service, C.D.C. Atlanta, Georgia.

Azizuddin, I.M. and Chandrasekhran Nair, K.P. (1954). Madras Vet.Coll.Annual, 12: 17-72. (Cited from Vet.Bull. 1971, pp. 176).

Ballenger, W.L. and Ballenger, H.C. (1943). Diseases of the nose, throat and ear. 8th Edn. Lea and Febiger. Philadelphia.

Baxter, M. and Lawler, D.C. (1972). Neuz.Vet.Jour., 20(3): 29-34.

Berg, O.A. (1951). Nord.Vet.Med., 3: 394.

Brumley, O.V. (1950). A Text Book of the Diseases of the small animals. Lea and Febiger, Philadelphia.

Cowan, S.T. and Steel, K.J. (1970). Manual For the Identification of Medical Bacteria. Published at the Cambridge University Press.

Cross, J.F. (1962). Aust. Vet.Jour., 38: 431-433.

Cruickshank, R. (1970). Medical Microbiology. A guide to the Lab. Diagnosis and control of infection. 11th Edn. The English Language Book Society and Els. Livingston Ltd.

Dam, A. (1962). Nord.Vet.Med., 4(12): 1207.

Dey, P.C., Tripathy, S.B., Ojha, S.C. and Mishra, S.K.(1972). Orissa Vet.J., 7(2 & 3): 89-92.

Enlows, E.M.A. (1935). M.Ann.District Columbia, 4: 217.

Farrag, H. and Hosny Mahmoud, A. (1953). Am.Vet.Med.Assoc., 122: 35.

Fraser, G. (1958). Personal communication. Cited by Joan, O. Joshua "Diseases of the External auditary meatus of the Dog and Cat (Vet.Rec.,70(49):1115-1125).

Fraser, G., Withers, H.R. and Spruell, J.S.A. (1961). J. Small Anim. Pract., 2: 32.

Fraser, G. (1961). J.Comp.Path., 71: 15.

Fraser, G. (1961a). Vet.Rec., 75: 3.

Fraser, G. (1961b). Vet.Rec., 53: 3.

Fraser, G. (1965). J.Small.Anim.Pract., 6: 445.

Frost, R.C. and Beresford Jones, W.P. (1958). Vet. Rec., 70: 740.

Frost, R.C. and Beresford Jones, W.P. (1960). Vet. Rec., 72: 375.

Grono, L.R. (1967). Ph.D. Thesis, University of Queensland, Australia. Cited by Sinha, B.K. (1970) in study of Otitis Externa in Dogs. Thesis submitted to the Faculty of All India Institute of Medical Sciences, New Delhi.

Grono,L.R. and Frost, A.J. (1969). Aust.Vet.Jour., 45(9): 420-422.

Grono, L.R. (1969a). Aust.Vet.Jour., 45(9): 417-419.

Grono, L.R. (1969b). Vet.Rec., 85: 6-8.

Grono, L.R. (1969). Vet.Rec., 85: 34.

Gustafson, B. (1944). Nord.Vet.Med., 6: 434.

Gustafson, B. (1955). Otitis externa in the Dog. Bacteriological and Experimental study, Stockholm. Cited by Sinha, B.K. (1970).

Hoffman, L. (1898). Osterr Monatschr. Tierheilk, 23: 193. (Cited by Gustafson, 1955).

Hugh, R. and Leifson, E. (1953). J.Bact., 66: 24.

Jacob, H. (1950). Ohrkrankheiten. Stang und Wirth Tierheilkunde und Tierzucht., 7: 557. Cited by Sinha, B.K. (1970).

Jennings, S. (1953). Vet.Rec., 65: 809.

Jones, W.G. (1955). J.Am.Vet.Med.Assoc., 127: 442-443.

Joshua, J.O. (1958). Vet.Rec., 70: 1115.

Kaplan, A.D. (1951). Vet.Med., 46(6): 212.

Kaufmann, E. and Frost, Ch. (1949). Vet.Rec., 61(26): 368.

Knowles, A.T., Knowles, J.O. and Knowles, R.P. (1918). North Amer.Vet., 29(8): 495.

Koutz, P. (1853). Vet. Med., 50: 278.

King,E.O.,Ward,M.K. and Raney,D.E.(1954). J.Lab.Clin.Med.,44:301.

Krall, P. (1936). Tierarztl. Wschr., 52(44): 712. Cited by Sinha, B.K. (1970).

Kral, F. (1957). J.Amer.Vet.Med.Assoc., 130: 41.

Mathew, Z., Murkibhavi, G.R. and Mathew, T. (1970). Ind.Vet. J., 47: 337-343.

McBride, N.L. (1953). Proc.Am.Vet.Med.Assoc. p. 247.

McGinnis, L. and England, R.B. (1949). Vet.Med., 44: 465.

Philips, S.E. (1953). The Ears. In Hoskins and Looroix. Canine Medicine. 2nd Edn. Amer.Vet.Publ.Inc. Santa Barbana.

Ridell, R.H. (1950). Slide Culture Technique. Cited by Ajello, L., Georg, L.K., Kaplan, W. and Kauffman, L. (1963). Lab.manual for Medical Mycol. Pub. Hlth. Ser. C.D.C., Atlanta, Georgia.

Riser, W.H. (1949). The Ears, Hoskins and Lacroix. Canine Surgery. Ivanstone, Ill.

Schoop, G. (1951). Deutsch. Tierarztl. Wschr., 58(27):216. Cited by Gustafson (1955).

Seibeuman, (1889). Quoted by Hikila. Jibiinkoka Rinsho (Otorhinolaryngological Clinic), 50:434, 1957.

Senturia, B.H. and Carr, C.D. (1958). Laryngoscope, 68:2052. Cited by Sinha, B.K. (1970).

Serth, G.W. (1954). Vet.Rec., 66(18): 254.

Singh, G.B. and Rao, M.M. (1959). Ind.Vet.Jour., 36(5): 236-242.

Salvin, S.B. and Lewis, M.L. (1946). J.Bact., 51: 495.

Sinha, B.K. (1970). Thesis submitted to the Faculty of All India Institute of Medical Sciences, New Delhi.

Smith, J.M.B. (1968). Aust.Vet.Jour., 44(9): 413-415.

Sutlie, A. (1939). Veterin. Archiv., 9(4): 253. Cited by Gustafson (1955).

Tufvesson, G. (1955). Am.J.Vet.Res., 55: 656.

Wagner, J.L., Pick, J.R. and Krigman, M.R. (1968). J.Am. Vet.Med.Assoc., 153: 945-949.

Wang, C.T. (1972). Memoirs of Coll. of Agri. National Taiwan University No. 2, 186-198.

Witter, B.E. (1949). Cornell Vet., 39: 1.

Zurn, F.A. Und Plaut, H. (1887). Weimar 306. Cited by Gustafson, 1955.