STUDIES ON THE STAPHYLOCOCCI OF CANINE ORIGIN WITH PARTICULAR REFERENCE TO THE CARRIER STATE OF PATHOGENIC STRAINS

by

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INTRODUCTION

Staphylococci are ubiquitous in nature and, therefore, staphylococcal infections attain worldwide distribution. The most important member of this genus is Staphylococcus aureus (Breed et al., 1957). Being an essentially pyogenic organism it is commonly associated with suppurative processes of varied intensity in man and animals. The infections include abscesses in any part of the body ranging from a small pimple on the skin to pyemia, septic complications and secondary abscesses by metastasis. When endocarditis is present it is frequently complicated by erosion and rupture of the valves with sudden myocardial failure and death. This organism is often the cause of purulent osteomyelitis. A fatal fulminant type of staphylococcal pneumonia followed the pandemic of influenza of 1917-1918. It has rightly been stated that staphylococci cause the most minor, most terrible and the most frequent bacterial infections.

The introduction of antibiotics has benefitted modern medicine in its struggle against staphylococcal infections but has brought in its wake the present day public health problem of antibiotic resistant strains, the development of which was probably inevitable. The medical profession was frequently conscious of the warnings put forth from time to time against the development of such antibiotic resistant strains on grounds that most antibiotics are bacteriostatic and not bactericidal and that sufficient concentration over long periods must be maintained in the body for effective bactericidal action. In actual clinical practice this is not possible for obvious reasons.

It can be reasonably assumed that the factors largely responsible for the development of antibiotic resistant strains may be one or more of the

following: (1) the frequent use of antibiotics in the treatment of febrile and other conditions of unproven etiology, (2) the tendency to relax aseptic precautions in hospital technique, relying too much on antibiotics to control secondary infection, and (3) the peculiar biologic properties of staphylococcal strains including an uncanny ability to become resistant to a wide range of antibiotics and therapeutic agents. This latter factor or property is probably responsible for the selection and propagation of the antibiotic resistant strains in places where antibiotics are intensively used in the treatment of disease.

The introduction of antibiotics and their effectiveness against bacteria in general and staphylococci in particular during the past two decades has reduced the impetus for fundamental research on the susceptible organisms so that the basic information about the ecology, pathogenesis, immunological relationships and mutability of staphylococci is lacking, compared to similar information available concerning other pathogenic organisms. Familiarity probably bred neglect of sufficient study of these organisms.

Primary septic involvements and secondary septic complications due to infection with staphylococci are at present bewildering the medical profession in many parts of the world. Relevant evidence has been presented concerning the widespread dissemination in the hospitals in the U.S.A. and also in other countries of certain antibiotic resistant strains of staphylococci with certain biologic properties which include high communicability from the carrier to the contacts, a tendency to produce nasal carriers, a tendency to produce skin lesions, septic complications of wounds, to invade tissues in sites of lowered resistance and a special propensity for developing resistance to antibiotic substances to combat them.

Staphylococci are spread from contaminated clothing, bed linen, air-dust and other fomites in the environment of patients. Such organisms resistant to several antibiotics and ubiquitous in nature offer a very serious problem in the control of transmission as well as the sources of infection.

Many hospitals today are determining the sub-clinical infection rate and distribution of resistant forms in their hospital staff by culture methods. Some are establishing the bacteriophage pattern of resistant strains of the organisms present in their hospital. Staphylococcal strains vary in the degree of virulence, and the biological characters of the organism which relate to their virulence are not well understood. The present day laboratory methods reveal no difference in virulence between pyogenic strains and coagulase positive strains from human carriers. However, clinical evidence shows that strains which are dispersed from open infections are more virulent than the strains of the same bacteriophage types carried in most of the healthy carriers.

It, therefore, suggests that new studies of immune and host-parasite relationships be carried out, based on the recent experience with staphylococci, because the present methods known to us may eventually fail to control infection caused by the strains which have already become established. The problem demands institution of all preventive measures against staphylococcus cross infection amenable for control at present and improved whenever better data becomes available. It is now necessary to know as much about these strains as possible because the mode of transmission is varied and complex and the organisms are common in nature. The chief source of danger is a carrier, human or animal. To what extent animal

carriers exist as reservoirs of infection is not definitely known. Information in this regard is very meager and that which is available is not satisfactory and has no relevance to the problem of the present day--the problem of epidemic strains of staphylococci.

Among the domesticated animals the canines occupy an unique position. Having been the first animal to be domesticated by man, the dog has served the human needs in a manner which cannot be emulated by any other animal. Primitive man used its flesh for food and its skin for clothes. The dog helped man in the chase and protected him and his goods from his enemies. Its intelligence and unstinted love and devotion towards its master, frought with obedience and implicit confidence and faith in him to its dying day, no matter what he is and who he is has obtained for it a status unsurpassed by other domesticated animals. A pet dog is a member of its master's household, fondled, caressed and with freedom of accessability to every part of the house.

Taking these things into consideration it was decided to make a survey of strains of staphylococci present on the skin and upper respiratory passages of dogs and determine their relationship to the typical epidemic strains of the organism.

It was thought that this investigation would provide information on whether or not these animals are frequent carriers of human strains of staphylococci. If a pet dog should harbor any of these human strains, transmission to human beings might easily be accomplished.

REVIEW OF LITERATURE

It was Robert Koch who attempted to determine whether or not the infective diseases of wounds of man were of parasitic origin; however, he was unable to procure the necessary clinical material for this specific investigation. Closely following Koch's staining technique, Ogston in 1880 examined the bacterial contents of 100 abscesses of varying severity. In all acute abscesses, he found cocci arranged both in clusters and in chains and succeeded in cultivating them by implanting them in fresh egg medium. With growths thus obtained, he produced abscesses in mice. He concluded that cocci produced inflammation and suppuration and pointed out that they may proliferate locally and invade the blood stream. He differentiated two kinds of cocci: (1) one arranged in chains corresponding to streptococci which had already been described and (2) the other arranged in masses to which he gave the name staphylococcus. Ogston's research soon came to exert its influence on the bacteriological, pathological, surgical and post-operative sequelae of inflammation and suppuration. Rosenbach, by means of gelatin cultures divided Ogston's staphylococci into two species which we know today and which have come to be reckoned as significant causative factors of disease and studied largely from this point of view (Bullock, 1930).

Mallais (1890) seemed to be the earliest investigator to isolate staphylococci from nasal mucosa, trachea and pneumonic lungs of dogs, while trying to evolve the cause of canine distemper. Heuer (1906) also isolated from the nasal discharge, pulmonary exudate and heart-blood of canines dead of distemper S. aureus and S. albus. Brumley (1938) had stated that the most common organisms besides streptococci found in the tonsils of dogs,

were staphylococci which multiplied rapidly in the tonsils bringing about tonsilitis and severe forms of pharyngitis. Stafseth (1940) examined the swabs from the tonsils of five apparently healthy puppies and found that three of them harbored "hemolytic" staphylococci, one hemolytic streptococcus and one both of these organisms. Bosworth (1947) while studying the possibility of staphylococcal tonsillar infections in canines in relation to chronic ill-health associated with mild widespread pustular dermatitis, had stated that staphylococci were not present as commonly in the tonsil swabs as streptococci but were only occasionally met with. Hemolytic strains of staphylococci had been isolated from throat swabs of dogs with enlarged pharyngeal and cervical lymphatic glands, and furunculosis and also occasionally from throat swabs in cases in which it seemed unlikely that the tonsil was playing any part in the disease observed. Under these circumstances it was premature to attach significance to the mere presence of staphylococci in the tonsils and also unwise to disregard them completely when they occurred in an obvious septic focus, with the involvement of the neighboring lymph glands.

A great deal of work had been devoted to the study of staphylococci from human sources in order to distinguish pathogenic from non-pathogenic strains. Several interesting and suggestive hypotheses had been propounded regarding the pathogenic part played by the cellular constituents, enzymes and products of metabolism of staphylococci, in the initiation of the infective process and the prognosis of the disease. Properties such as pigment production, fermentation of mannitol, liquefaction of gelatin, the production of hemolysis on blood agar and production of filterable toxins had all been examined. The formation of leucocidin, fibrinolysin and the

lethal effect of the organisms themselves when injected into experimental laboratory animals had also been studied.

Work on the classification of staphylococci, in particular, the investigations by Chapman et al. (1934) Cruickshank (1937), Fairbrother (1940) and Christie and Keogh (1940) had shown that while these tests are of considerable value in the determination of pathogenicity, the absolute single criterion of pathogenicity is the coagulase production.

A search of the literature revealed that until recently very little of the work done on staphylococci of animal origin had been directed towards determination of criteria of pathogenicity.

Minett (1936) examined 51 pathogenic strains from various animal sources with particular reference to their toxin production and not coagulase production. He concluded that the production of B-toxin was a characteristic feature of hemolytic staphylococci obtained from animals and that strains from dogs could be differentiated by their greater proteolytic power.

Cruickshank (1937) examined six animal strains of unspecified origin for coagulase production by the tube test and all were coagulase positive.

Bell (1940) studied 10 strains isolated from various pyogenic processes from dogs for production of pigment, toxins, hemolysis and coagulase and mannitol fermentation. He concluded that coagulase production more nearly correlated with hemolysis production, than did fermentation of mannitol.

Field and Smith (1945) examined a large number of strains of staphylococci of human and animal origin and concluded that coagulase production was confined to pathogenic staphylococci.

Christie et al. (1946) tested over 1000 strains of this organism from diverse animal sources for coagulase production, hemolysis on sheep blood agar, pigment production, fermentation of mannitol and fibrinolysin. Pathogenicity tests with mice were also conducted. The authors came to the conclusion that a strain may produce coagulase and still be non-pathogenic and that all pathogenic strains produce hemolysis on sheep blood agar while non-pathogenic strains do not.

Smith (1947) in his study of 39 canine strains of staphylococci for various properties, including dermotoxin production and lethal effect of toxin and the organisms on mice and rabbits, concluded that the production of coagulase was the only absolute criterion of pathogenicity. Few of the pathogenic strains showed in addition liquefaction of solid serum or fibrin or production of Beta hemolysis on sheep blood agar. It was his opinion that pathogenic strains from dogs formed a fairly distinct group. The cultures all produced white colonies, formed much Beta toxin, little or no Alpha toxin and were non-lethal to mice and rabbits. Most of the strains actively liquified solid serum and all produced fibrinolysin.

Shetty (1948) estimated that 49.3 per cent of the normal dogs and 68 per cent of the sick dogs examined by him at the clinics Royal (Dick) Veterinary College, Edinburgh, harbored coagulase positive staphylococci in their upper respiratory tracts. The observation compared favorably with the findings of Smith (1947).

Dumas (1914) examined the cultural and biochemical reactions of 17 strains, out of which eight were of animal origin.

Bacteriophage Typing. Twort in 1915 and d'Herelle in 1917 observed bacterial lysis by a transmissible lytic agent. This lytic agent which

which caused lysis of the bacterial cells and known as Bacteriophage (or more simply, "phage") was ultra-microscopic and filterable. The lysis was transmissible in series and was generally known as Twort-d'Herelle's phenomenon or more simply lysis by bacteriophage.

d'Herelle in the period from 1921 to 1926 attempted to implicate bacteriophage in the phenomenon of recovery from all types of infectious diseases. The evidence available did not support his attempt at implication. Phage therapy in the form of enteral and parenteral administration in enteric and a variety of other infections was disappointing. But one of the important features of d'Herelle's work was the development of a quantitative approach using both lysis in broth and the production of discrete clearings or plaques on confluent surface bacterial growth on agar (Burnett, 1955).

Phage strains that can establish a lysogenic relation with bacteria are called temperate phages. Strains that do not establish lysogenicity but regularly lyse the bacterial cells they infect are called virulent phages. As a rule only bacteria that can grow a phage will absorb it. In some cases the phage can be absorbed by bacteria unable to support its growth and become lysed. Serological cross reactions have been found between the normal host for a phage and the non-host bacteria that adsorb the phage suggesting a role of antigenic constitution of the bacterial surface in phage adsorption. There is a fairly good relation between possession of certain antigens and sensitivity to phages (Luria, 1956). Sensitivity to bacteriophages had been used as an aid in the classification and differentiation of individual strains of several bacterial species and the bacteriophages had become useful adjuncts for taxonomic and epidemiological

studies of bacterial infections in view of their definite selective action against strains of bacteria of the same species. Furthermore, the susceptibility of particular strains of an organism to the action of the specific phages and the specific action of the phage are constant and stable characteristics (Blair, 1956).

Williams and Timmins (1938) appear to be the earliest workers to use bacteriophages in an attempt to distinguish various strains of staphylococci by testing broth cultures for their susceptibility to the series of phages isolated by Burnet and Lush in 1935. (Anderson and Williams, 1956).

Fisk (1942) described the cross-culture method for the detection of lysogenic strains of staphylococci and identified 24 different phages. The incidence of lysogens in this study with 43 strains was 44.2 per cent. Further study with 95 strains showed the differential action of these phages and made it possible to divide cultures of pathogenic staphylococci into groups and to recognize a "susceptibility pattern." By comparing the pattern, similarity or dissimilarity of two strains could be determined. Strains isolated from the related sources were found to react to the same phages and could be differentiated from other strains by this method. Fisk's method involved growing two strains of staphylococci, one superimposed over the other. If any of these two strains carried a latent phage to which the other was susceptible, appearance of plaques would be indicative. The phage was then isolated by growing on each of the pair of strains involved and propagated by serial passages on agar cultures of the susceptible strain.

Wilson and Atkinson (1945) modified Fisk's method to permit the differentiation of staphylococci in a manner comparable to that which had been

successfully used by Craigie et al. (1938) in phage typing of Salmonella typhosa. One important modification was the introduction of a "Routine Test Dilution" for each staphylococcal phage. The routine test dilution (R.T.D.) was defined as the highest dilution of the phage which produced confluent lysis of its propagating strain on agar. Dilutions higher than this produced either semi-confluent or isolated plaques. The test dilution of each phage was characteristic for that phage and remains fairly constant with minor variations above or below that titre, as each successive new lot of phage was prepared. The greatest landmark in the routine phage typing program and epidemiological study was the establishment of a Staphylococcus Reference Laboratory for the Public Health Service under Dr. V. D. Allison in 1946.

Blair and Carr (1953) outlined a phage typing method for coagulase positive staphylococci based on methods described by Fisk (1942) and by Wilson and Atkinson (1945), which is being conducted in most laboratories in the U.S.A. engaged in phage typing. The Communicable Disease Center, which is the official typing center for the U.S.A. slightly modified it. The International Sub-Committee on Staphylococcal Bacteriophage Typing, which met at Stockholm in August, 1958 further modified it with regard to the basic set of phages chosen for the routine phage typing work.

Human and Animal Staphylococci. Applying the phage typing method devised by Wilson and Atkinson (1945), William Smith (1948) studied the epidemiology of staphylococcal infections of the udder in cattle. He found that a high proportion of coagulase positive bovine strains were susceptible to one phage--phage 42D. Investigations conducted earlier by Macdonald (1946) had yielded similar results. Price et al. (1956) found in addition

to 42D phage strains, staphylococci of Group III were common (phage 42D has now been allocated to Group IV). Strains lysed by phages belonging to Groups I and II were not uncommon. Some strains were practically lysed by all the phages (Anderson and William, 1956). Apart from bovine strains, canine strains of staphylococci were more insensitive to typing phages than human strains (Levy Rippon and Williams, 1953).

Roundtree et al. (1956) examined nasal swabs and found seven carriers among 35 hospitalized dogs belonging to the following phage patterns while no carriers were found in 26 out patients.

No. of strains	phage pattern
2	7/42 G/47.
1	7/47 D.
2	31 B/44.
1	42 G/47
1	N. T.

Coles (1958) did not find typeable strains among the strains of staphylococci isolated from canines and studied by him.

MATERIALS AND METHODS

The upper respiratory tract and the normal skin of dogs were examined for the presence of staphylococci. The canines visiting the Dykstra Veterinary Hospital were the source of the material for examination. Both normal and sick dogs were utilized.

With the help of an assistant to hold the dog with its head raised, a sterile cotton swab was passed gently through one nostril, after cleaning the exterior, into the nasal cavity as far as it is conveniently possible

without causing discomfort to the animal. The swab was withdrawn and passed into the other nostril in the same way. Separate sterile swabs were used in some cases. It was not possible to pass the swab far inside the nasal cavity; nevertheless, sufficient mucus was always available on the swab. Similarly while the assistant opened the mouth of the dog, the throat and tonsils were touched with a sterile swab and satisfactory material obtained. Most of the animals were hospitalized for treatment. To save them from further distress swabbing the naso-pharynx was avoided.

Hairless portions of the skin such as, axilla and groins were swabbed to study the occurrence of staphylococci on the skin.

Bacto-Staphylococcus medium No. 110, a selective medium for the isolation of staphylococci, was used for the primary isolation of staphylococci from the various swabs obtained throughout the investigation.

Each swab was smeared over a small area of the culture plates and streak isolations were made for obtaining discreet colonies. The plates were examined after sufficient incubation at 37° C. for colonies resembling staphylococci. The colonies were observed for evidence of pigmentation tested for purity and staining reaction with Gram's method and picked onto agar slopes for further study. They were also grown anaerobically by means of stab inoculations in tubes of proteose peptone agar containing 0.1% glucose.

A drop of Brom-cresol purple indicator was added to the area on Staphylococcus medium No. 110, from which the typical colonies were removed. Any change in color of the indicator towards yellow compared with its normal color was taken to indicate the fermentation of mannitol. The plate was then flooded with 5 ml. of saturated solution of ammonium sulphate and

allowed to stand for 10 minutes. Clear zones, around the areas from which colonies have been removed, with an opaque white background indicated a positive gelatinase reaction.

The coagulase test was performed on all isolated staphylococci. For the coagulase test 24-hour-old cultures of the organisms on agar slants were used, and coagulase production in rabbit and human plasma determined. For this purpose a small loopful of the culture was added to 0.5 ml. of reconstituted Bacto-coagulase rabbit plasma and another similar loopful to 0.5 cc. of citrated human plasma in sterile Wasserman tubes. The tubes were incubated in a water bath at 37° C. Readings were made at the end of 1, 3, and 24 hours' incubation. Any degree of clotting during the period, however slight, was considered positive.

The pattern of hemolysis on rabbit, sheep and human red blood cells was studied by growing the various strains of the organism on sterile agar containing five per cent sterile defibrinated rabbit, sheep and human blood, respectively. Sensitivity to antibiotics was determined by growing the various strains separately on proteose peptone agar plates in the presence of antibiotic sensitivity discs.

Bacteriophage typing of coagulase positive strains was carried out by the use of human phages infective to human S. aureus strains to determine the phage relationship of strains of the organism harbored by canines.

The various coagulase positive strains of staphylococci that were isolated and studied as already reported in the earlier part of this thesis were subjected to each of the 20 human staphylococcal phages maintained for use at the Kansas State Board of Health Laboratory, Topeka, Kansas, in their routine phage typing work. These 20 phages constituted the basic set

chosen by the International Sub-Committee on Staphylococcal Bacteriophage Typing which met at Stockholm in August, 1958. They were grouped in the following manner.

Group I	29, 52, 52A, 79, 80.
Group II	3A, 3B, 3C, 55, 71.
Group III	6, 7, 42E, 47, 53, 54, 73, 75, 77.
Group IV	42D.
Miscellaneous	81

The committee mentioned above had recommended that in addition to the basic set of phages other phages might be used at the discretion of the investigator, if he should feel that such usage would provide useful information.

Technique of Phage Propagation. Trypticase soy agar and broth (Baltimore Biological Laboratory) and 300 ml. prescription bottles with screw caps were used.

For each phage to be prepared, about 30 ml. of 1.5 per cent trypticase soy agar were poured into a prescription bottle and layered on the flat side.

A four-hour-old trypticase soy broth culture of the host strain of the phage to be propagated was prepared. Three-tenths ml. of the broth culture and 0.275 ml. of the undiluted phage were measured into a sterile test tube. Ten ml. of 0.7 per cent trypticase soy agar were melted and cooled to 45° C. and added to the staphylococcus-phage mixture, which was then layered over the 1.5 per cent trypticase soy agar in the prescription bottle and allowed to harden. The bottle was incubated at 35° C. overnight.

The soft agar (0.7%) layer was harvested into a centrifuge tube and the bottle was rinsed with 10 ml. of trypticase soy broth and vigorously

shaken and transferred into the centrifuge tube. The harvested agar and broth washing were shaken to break the agar and release the phage into the suspension. The mixture was centrifuged at 3000 rpm. for 30 minutes. The supernatant broth containing the phage was pipetted off. This process was repeated until the phage titre was high enough to use. The phage thus obtained was stored at 4° C. in screw-capped bottles.

Titration. It was important that the newly propagated phage should be of sufficient titre. Tripticase soy agar plates were poured and incubated at 35° C. for 48 hours to dry. They were stored at room temperature until required for use.

A four-hour trypticase soy broth culture of the propagating staphylococcal strain of the phage to be titrated was prepared.

Serial 10-fold dilutions of the phage in trypticase soy broth were made from 10^{-1} to 10^{-6}. Separate pipettes were used for each dilution.

The trypticase soy agar plate was seeded with the broth culture by means of a sterile swab without disturbing the surface of agar. After allowing it to dry, the agar plate was inoculated with the 10-fold dilutions of the phage and incubated at 35° C. for four hours and allowed to stand at room temperature overnight. The Critical Test Dilution (C.T.D.) was the highest dilution producing completely confluent lysis. It is said that most staphylococcal phages would give titres of 10^{-4} to 10^{-6} and frequently higher, but the highest titre that could be obtained has not yet been determined. Before use, in routine tests, each lot of each phage was tested on all the propagating strains to determine its pattern of activity as a guard against changes and/or contamination of phages during propagation, the C.T.D. being used for the purpose.

Typing the Unknown. Permanent red 30 square grid petri plates were available for use. One and five tenths per cent trypticase soy agar plates were poured and dried in the incubator at 35° C. for 48 hours. One plate was required per culture to be typed. Four-hour-old trypticase soy broth cultures of the strains of staphylococci to be typed and also all the propagating strains were prepared. Using a sterile swab the strains of staphylococci to be typed were seeded on trypticase soy agar plates and allowed to dry under the table lamp. They were labeled at the top of the grid pattern.

Each phage was diluted to its C.T.D. A pattern of phage inoculation was prepared as shown in the diagram.

One drop of each phage from a loaded syringe with 27 gauge needle was discharged in the appropriate square of one plate and proceeded to the next and so on until all the plates were inoculated. The process was repeated with all phages, in the order shown in the diagrams in Figures 1 and 2.

One drop of each phage at C.T.D. was set up, with its propagating strain of staphylococcus as control to guard against drops in titre. The plates including the controls were incubated at 35° C. for four hours and held at room temperature overnight. The patterns were read using a bright light against a black background:

Confluent lysis	+ + +
50 plaques and over	+ +
20-50 plaques	+
less than 20 plaques	±

29	52	52A	79	80	3A
3B	3C	55	6	7	42E
47	53	54	73	75	77
42D	81				

Fig. 1 Design followed for phage inoculation in 30 grid petri dish.

3A	80	79	52A	52	29
42E	7	6	55	3C	3B
77	75	73	54	53	47
				81	42D

Fig. 2 Design of inoculated phages on the inverted plate.

As most of the cultures did not type at C.T.D., each culture was set up with a concentration of 1000 × C.T.D. or a concentration of 10^{-1} dilution.

Only + + + and + + reactions are reported as a part of the "phage pattern" of the strain.

RESULTS

Facultatively anaerobic, Gram positive, non-motile spherical organisms appearing in irregular clusters were identified as staphylococcus species (Breed et al., 1957).

Staphylococci were isolated from 30 (93.75%) of 32 dogs examined, from one or more situations. Twenty-eight dogs (87.5%) carried coagulase positive strains of staphylococci. The frequency of their occurrence in the anterior part of the nares, throat and the selected hairless locations on the skin are shown in Table 1. The incidence of staphylococci in general and the coagulase positive strains in particular appear to be very high in the nose, as compared with other locations. Therefore, it may be presumed that the anterior nares are more important as a reservoir of staphylococci in dogs. Though the incidence of occurrence of strains of staphylococci was the same both in the axillary region and in the groin, the incidence of coagulase positive strains in the groin was greater (Table 2).

Table 1. Carrier rate of staphylococci.

Number of dogs examined	Number which harbored staphylococci	Per cent	Number of dogs which harbored coagulase-positive staphylococci	Per cent
32	30	93.75	28	87.5

Table 2. Frequency of distribution of staphylococci.

Location	Number of swabs examined	Number of swabs from which staphylococci were isolated	Per cent	Number of swabs which yielded coagulase positive strains	Per cent
Throat	31	14	45.2	12	38.7
Nose	30	25	83.3	21	70.0
Skin					
(a) Axilla	28	13	46.4	6	21.4
(b) Groin	28	13	46.4	11	39.3

Coagulase Production. Of the 65 strains examined for coagulase production using both citrated human and rabbit plasma, 49 (75.4%) strains coagulated human plasma and 46 (70.8%) coagulated rabbit plasma. The coagulase activity of the 65 strains is shown below.

38 strains coagulated both human and rabbit plasma.

11 strains coagulated human plasma alone

8 strains coagulated rabbit plasma.

A greater proportion of coagulase positive strains were isolated from the nose (Table 3).

Pigment Production. Fourteen of the 65 strains (21.5%) produced the typical golden yellow or orange pigment of Staphylococcus aureus on Staphylococcal medium 110, while the rest were white (Table 3). Seven (50%) of the 14 strains occurred in the nose. Of the 57 coagulase positive strains 13 produced the aureus pigment (22.8%). This is in agreement with the observations made by Smith (1947) that only a minor percentage of pathogenic staphylococci of dogs form aureus pigment. Only one out of eight coagulase negative strains produced aureus pigment (Table 4). The differential reactions between the aureus-pigmented strains and white pigmented strains are shown in Table 5. A consistently high percentage of the aureus-pigmented

Table 3. Summary of data of characteristics of strains of staphylococci.

Location	Number of strains isolated	Coagulase production on human plasma	Coagulase production on rabbit plasma	Pigment production: orange	Pigment production: white	Mannitol fermentation	Gelatin liquefaction	Hemolysis on human RBCs	Hemolysis on sheep RBCs: Alpha	Hemolysis on sheep RBCs: Beta	Hemolysis on sheep RBCs: Cold	Hemolysis on rabbit RBCs
Throat	14	11	12	4	10	1	5	10	1	4	3	5
Percentage		78.5	85.7	28.6	71.4	7.1	35.7	71.4	7.1	28.6	21.4	35.7
Nose	25	21	19	7	18	6	10	16	2	8	6	9
Percentage		84.0	76.0	28.0	72.0	24.0	40.0	64.0	8.0	32.0	24.0	36.0
Skin:												
Axilla	13	6	6	2	11	3	4	5	1	2	1	2
Percentage		46.2	46.2	15.3	84.6	23.1	30.8	38.5	7.7	15.4	7.7	15.4
Groin	13	11	9	1	12	-	3	6	-	2	2	-
Percentage		84.6	69.2	7.7	92.3	-	23.1	46.2	-	15.4	15.4	-
Total	65	49	46	14	51	10	22	37	4	16	12	16
Percentage		75.4	70.8	21.5	78.5	15.4	33.8	56.9	6.2	24.9	18.5	24.9

Table 4. Differential reactions between coagulase positive and coagulase negative strains.

Reactions	57 Coagulase positive strains: Positive	Negative	Per cent positive	8 Coagulase negative strains: Positive	Negative	Per cent positive
Pigmentation	13 (orange)	44 (white)	22.8	1 (orange)	7 (white)	12.5
Mannitol fermentation	10	47	17.5	-	8	--
Gelatin liquefaction	19	38	33.3	3	5	37.5
Hemolysis on sheep blood agar:						
(Semi-clear) Alpha	4	53	7.0	-	8	--
(Clear) Beta	14	43	24.6	2	6	25.0
Cold lysis	11	46	19.3	1	7	12.5
Hemolysis on human blood agar	34	23	59.6	3	5	37.5
Hemolysis on rabbit blood agar	15	42	26.3	1	7	12.5

strains gave positive reactions. Among the white-pigmented strains a high percentage caused hemolysis of human blood and coagulated rabbit and human plasma (Table 5).

Mannitol Fermentation. Of the 65 strains, 10 (15.4%) fermented mannitol. Six of these 10 strains were isolated from the nose (Table 3). All 10 strains were coagulase positive. None of the coagulase negative strains fermented this sugar. The remaining 47 coagulase positive strains did not ferment mannitol (Table 4). These results indicate that mannitol fermentation does not serve as a criterion to classify pathogenic strains in dogs. This is in agreement with the findings of Cowan (1939) who stated that greater correlation between mannitol fermentation and coagulase

Table 5. Differential reactions between orange pigmented and white strains of staphylococci.

Reactions	14 orange pigmented strains: Positive	Negative	Per cent positive	51 white strains: Positive	Negative	Per cent positive
Mannitol fermentation	7	7	50.0	3	48	5.9
Gelatin liquefaction	11	3	78.6	11	40	21.6
Hemolysis on human blood agar	12	2	85.7	25	26	49.0
Hemolysis on sheep blood agar:						
(Semi-clear) Alpha	2	12	14.3	2	49	3.9
(Clear) Beta	9	5	64.3	7	44	13.7
Cold lysis	6	8	42.9	6	45	11.8
Hemolysis on rabbit blood agar	9	5	64.3	7	44	13.7
Coagulase production:						
(a) Rabbit plasma	12	2	85.0	34	17	66.7
(b) Human plasma	11	3	78.6	38	13	74.5

production existed among strains of human origin than in those of animal origin. Bell (1940) and Smith (1947) arrived at similar conclusions.

Gelatin Liquefaction. Of the 65 strains, 22 (33.8%) liquefied gelatin (Table 3). Ten of these were isolated from the nose. Nineteen (33.3%) of the 57 coagulase positive strains liquefied gelatin. Three (37.5%) of eight coagulase negative strains also produced this reaction (Table 4). There is no correlation between coagulase production and gelatin liquefaction, which finding is in agreement with those of Smith (1947). This test is therefore of no value in differentiating pathogenic strains from non-pathogenic strains from canines. However, there appears to be a correlation between gelatin liquefaction and production of aureus pigment.

Hemolysis. Thirty-seven (56%) of the 65 strains examined produced hemolysis on human blood agar plates (Table 3). Thirty-four of these were coagulase positive and three coagulase negative; 59.6 per cent of the coagulase positive strains and 37.5 per cent of the coagulase negative strains produced hemolysis on human blood agar (Table 4). The correlation between aureus pigment production and hemolysis also seemed to be high (Table 5).

On rabbit-blood agar plates, 16 (24.9%) of the 65 strains produced hemolysis (Table 3), 15 (26.3%) strains were coagulase positive and one was coagulase negative (Table 4).

Storage of these plates at 4° C. for 24 hours widened the zones of hemolysis.

Hemolysis on sheep-blood agar plates were of two types: (a) semi-clear zones of hemolysis and (b) clear zones, when plates were incubated at 37° C. for 24 hours, which types, for the purpose of this classification, were termed Alpha and Beta hemolysis respectively. Subsequent storage of these plates at 4° C. for 24 hours exhibited in a few cases semi-clear zones around the hemolytic zones already obtained at 37° C. This type of hemolysis was termed 'cold hemolysis'. Of the 65 strains, four (6.2%) showed Alpha type, 16 (24.9%) Beta type and 12 (18.5%) cold hemolysis (Table 3). The relation between coagulase production and hemolysis on sheep-blood agar seems to be of an inconstant character (Table 4) as 64.3 per cent of the aureus pigmented strains produced Beta hemolysis (Table 5), 14.3 per cent Alpha variety and 42.9 per cent cold hemolysis.

In summary, Alpha and cold hemolysis were produced by four strains, Beta hemolysis alone was produced by nine strains; Beta and cold hemolysis were produced by seven strains and cold hemolysis alone was produced by one strain.

The hemolytic activity, in general, of all the strains isolated is summarized as follows:

11 strains lysed human, sheep and rabbit erythrocytes (29.7%),

10 strains lysed human and sheep erythrocytes (27.1%),

5 strains lysed human and rabbit red blood cells (13.5%) and

11 strains lysed human red blood cells alone (29.7%).

Fifty seven coagulase positive strains were subjected to the action of the basic set of human typing phages recommended by the International Sub-Committee on Staphylococcal Bacteriophage Typing (Stockholm, August, 1958).

Nineteen of the 57 strains could be typed. The typing reactions are shown in Table 6 and the phage pattern separately in Table 7.

It was found that of these 19 typeable strains studied in this investigation, 14 were typeable wholly or predominantly by phages belonging to Group III; phage 77 seemed to be the predominating single phage involved in 11 typing reactions.

Antibiotic resistance of the typeable strains was determined by growing each of these typeable strains in the presence of antibiotic sensitivity discs. The following antibiotics were used:

(1) Penicillin (2) Dihydrostreptomycin.

(3) Aureomycin (4) Neomycin (5) Erythromycin.

(6) Bacitracin (7) Furadantin (8) Chloromycetin

(9) Kantrex (Kanamycin).

Of these 19 strains, 14 showed resistance to varied numbers of antibiotics (Table 8).

The antibiotic resistance of the 19 typeable strains showing their phage groups, places from which they were isolated, and the resistance pattern are shown in Table 9.

Table 6. Phage-typing Reactions.

Culture number	C.T.D.	Concentration (1000 × C.T.D.)	Concentration (1/10 dil.)
17 N	3B(±)	3B(2+)	
5190 T	N. T.	53(1+)	53(2+)
5882 N	81(±)	81(2+)	
5636 A	77(1+)	53(1+) 77(2+)	
2710 N	53(±) 77(±)	53(2+) 77(2+)	
2710 T	7(2+) 47(±) 77(±) 81(±)	6(2+), 7(2+) 42E(2+) 47(2+) 53(2+), 54(2+) 75(2+) 81(2+)	
5636 N	77(±)	47(±) 53(±) 77(2+)	
5938 N	77(±)	52(2+) 77(2+)	
5032 N	29(±)	29(2+) 42E(2+) 53(2+)	
K2/2 G	N. T.	55(1+)	3B(2+) 3C(2+) 55(2+)
K2/2 A	3B(±) 3C(±)	3B(2+) 3C(2+)	
5938 T	77(±)	53(±) 77(2+)	53(2+) 77(2+)
5882 T	77(±)	53(2+) 77(2+)	
5071 T			
A	77(±)	53(1+) 77(2+)	
B	7(2+) 77(±)	6(2+), 7(2+), 42E(2+), 47(2+) 53(1+), 54(1+), 77(2+), 42D(2+) 81(2+)	
5232 T	29(±)	29(2+) 3A(±) 42E(2+) 47(±) 53(±), 54(1+) 75(1+)	
K 2/4 T	N. T.	N. T.	3B(2+), 3C(2+) 55(2+)
5439 N	77(±)	53(±) 77(2+)	
7153 N	77(±)	53(2+) 77(2+)	

Table 7. Phage patterns.

Culture number	Phage pattern
17 N	3B
5190 T	53
5882 N	81
5636 A	77
2710 N	53, 77
2710 T	6, 7, 42E, 47, 53, 54, 75, 77, 81
5636 N	77
5938 N	52, 77
5032 N	29, 42E
K 2/2 G	3B, 3C, 55
K 2/2 A	3B, 3C
5938 T	53, 77
5882 T	53, 77
5071 T	
A	77
B	6, 7, 42E, 47, 77, 42D, 81
5032 T	29, 42E
K 2/4 T	3B, 3C, 55
5439 N	77
7153 N	53, 77

Table 8. Antibiotic resistance of the typeable strains of staphylococci.

Case number and location	Antibiotics* 1	2	3	4	5	6	7	8	9
17 N	V.S	V.S	V.S	V.S	V.S	S	V.S	V.S	V.S
5190 T	R	R	R	S	R	V.S	V.S	S	V.S
5882 N	R	R	S.S	V.S	V.S	S	V.S	V.S	V.S
5636 A	R	R	R	V.S	V.S	S	V.S	S	V.S
2710 N	R	R	R	V.S	V.S	S	V.S	S	V.S
2710 T	R	R	R	S	V.S	S	V.S	S	V.S
5636 N	R	R	R	V.S	V.S	V.S	V.S	V.S	V.S
5938 N	R	R	R	V.S	V.S	V.S	V.S	S	V.S
5032 N	S.S	S.S	V.S	V.S	V.S	V.S	V.S	V.S	V.S
K 2/2 G	V.S	V.S	V.S	V.S	V.S	V.S	V.S	V.S	V.S
K 2/2 A	R	R	V.S	V.S	V.S	S	V.S	V.S	V.S
5938 T	R	R	R	V.S	S	S	V.S	S	V.S
5882 T	R	R	R	S	V.S	S	V.S	S	V.S
5071 T A	S.S	R	R	S	V.S	S	V.S	S.S	V.S
B	S.S	R	R	S	V.S	S	V.S	S.S	V.S
5032 T	S.S	S.S	V.S	V.S	V.S	V.S	V.S	S	V.S
K 2/4 T	V.S	V.S	V.S	V.S	V.S	V.S	V.S	V.S	V.S
5439 N	R	R	R	V.S	V.S	V.S	V.S	S	V.S
7153 N	R	R	R	R	S	S	S	S	V.S

* 1 - Penicillin (5 units)
2 - Dihydrostreptomycin (10 mg)
3 - Aureomycin (10 mcg)
4 - Neomycin (10 mcg)
5 - Erythromycin (5 mcg)
6 - Bacitracin (10 units)
7 - Furadantin (150 mcg)
8 - Chloromycetin (10 mcg)
9 - Kantrex (Kanamycin) (30 mcg)

R = Resistant
S = Sensitive
S.S= Slightly sensitive
V.S= Very sensitive

Table 9. Antibiotic Resistance of 19 phage typeable staphylococcus cultures. (Resistance Pattern).

Phage group	Isolated from T N A G *	Resistant to antibiotic **
III	3 4 1 0	1, 2, 3
	0 1 0 0	1, 2, 3, 4
	1 0 0 0	1, 2, 3, 5
	2 0 0 0	2, 3
	1 1 0 0	(Sensitive to all)
II	1 1 0 1	(Sensitive to all)
	0 0 1 0	2, 3
	0 0 1 0	1, 2
Misc. (Type 81)	0 0 1 0	1, 2

* Area of Isolation

T = Throat
N = Nose
A = Axilla
G = Groin

** Antibiotics:

1 - Penicillin (5 units)
2 - Dihydrostreptomycin (10 mcg)
3 - Aureomycin (10 mcg)
4 - Neomycin (10 mcg)
5 - Erythromycin (5 mcg)
6 - Bacitracin (10 mcg)
7 - Furadantin (150 mcg)
8 - Chloromycetin (10 mcg)
9 - Kanamycin (30 mcg)

SUMMARY AND CONCLUSIONS

The results of this investigation clearly showed that the incidence of staphylococci in canines is high, both in the upper respiratory tract and on the skin. Staphylococci were isolated from 30 (93.75%) of 32 dogs examined from one or more areas of the body. Twenty-eight dogs (87.5%) carried coagulase positive strains of staphylococci. The incidence of these organisms in general and that of the coagulase positive strains in particular, was found to be higher in certain locations. The skin of the groin was found to harbor a higher percentage of coagulase positive strains than that of the axilla.

The strains isolated appeared to vary in their capacity to coagulate rabbit and human plasma. Some strains which coagulated rabbit plasma did not produce the same reaction in human plasma and vice versa. It is, therefore, logical that strains of staphylococci should be tested for coagulase production both in rabbit and human plasma before they are declared as coagulase negative.

The typical orange pigment was produced by a very small number of strains of staphylococci isolated. Only 14 (21.5%) of the 65 strains examined produced this pigment. These strains showed a higher percentage of positive reactions to the usual tests for pathogenicity.

Mannitol fermentation and gelatin liquefaction were produced by very few strains, perhaps not of sufficient frequency as to be considered of any value in determining pathogenicity.

Coagulase positive strains and coagulase negative strains liquefied gelatin in almost equal numbers, though these were few. Therefore, this test cannot be of value as an index of pathogenicity.

The results of hemolysis tests on human, rabbit and sheep red blood cells showed that human red cells were lysed by the largest number of strains while a smaller number lysed rabbit red cells. The correlation between coagulase production and hemolysis of human red blood cells appeared to be significant.

It would, therefore, appear that in addition to coagulase production, hemolysis of human red blood cells may be used as an ancillary test for the determination of pathogenicity.

Fifty-seven coagulase positive strains were subjected to the action of the basic set of human typing phages recommended by the International Sub-Committee on Staphylococcal Bacteriophage Typing, Stockholm, August 1958.

Nineteen of the 57 strains could be phage typed. The results showed that these 19 strains could be identified as belonging to phage patterns characteristic of human strains. It is not possible at this stage to decide whether these strains with their phage patterns could be regarded as canine strains of the organism. It is reasonable to assume that these animals might have acquired their strains by close contact with human beings.

It is becoming increasingly evident that certain phage types of staphylococci are more or less characteristic of particular infections. Allison (1949) and Allison et al. (1949) found that the staphylococci responsible for outbreaks of food poisoning were lysed by phages 6 and 47. Later work has shown that food poisoning staphylococci belong to the broad phage Group III (William et al., 1953). Based on this accumulating evidence and in the absence of a satisfactory test for the detection of enterotoxin, it is the opinion of workers in Britain that the food poisoning strains almost

always belong to phage Group III (Anderson and Williams, 1956).

Of the 19 typeable strains studied in this investigation, 14 were typeable wholly or predominantly by phages belonging to Group III. Phage 77 was the predominating single phage involved in 11 typing reactions.

Of these 14 strains typeable by phages of Group III, 12 showed remarkable resistance to broad spectrum antibiotics. This association of Group III strains with resistance to broad spectrum antibiotics had already been recognized in "hospital infections", i.e., epidemic strains (Anderson and Williams, 1956; Blair and Carr, 1958).

Taking all these observations into consideration it is inferred that pet dogs are capable of harboring human epidemic strains of staphylococci with justifiable certainty of their possible transmission among the members of their masters' household.

Levy et al. (1953) had stated that animal strains, other than bovine were insensitive to human typing phages while Rountree et al. (1956) isolated six strains from the anterior nares of dogs that could be typed. Coles (1958) did not find typeable strains among the strains of staphylococci isolated from canines and studied by him. Coles' observations combined with the present investigation strengthen the belief in the carrier state in canines. The species of bacteria isolated from the upper respiratory tract and skin vary from place to place and from time to time so that one observer may record a high incidence of a particular bacterium and another worker other bacterial species.

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APPENDIX

Table 10. Characteristics of strains of staphylococci isolated.

Culture number	Location	Pigment	Mannitol fermentation	Gelatin liquefaction	Hemolysis on human blood agar	Hemolysis on sheep blood agar: Alpha	Beta	Cold	Hemolysis on rabbit blood agar	Coagulase production on human plasma	Coagulase production on rabbit plasma
5882	T	O	+	+	−				−	−	+
	N	W	−	−	−				−	+	+
	A										
	G										
2710	T	O	−	+	+		+		+	+	+
	N	O	−	+	+		+		+	+	+
	A										
	G										
5032	T	W	−	−	+				−	−	+
	N	O	+	+	+		+	+	+	+	+
	A										
	G										
4969	T										
	N	O	−	−	+		+	+	−	+	−
	A										
	G										
5890	T										
	N	W	−	−	−				−	−	−
	A										
	G										
4962	T										
(17)	N	O	−	+	+	+		+	−	+	+
	A	W	+	−	−				−	−	−
	G	W	−	−	+				−	+	+

Table 10. (contd)

Culture number	Location	Pigment	Mannitol fermentation	Gelatin liquefaction	Hemolysis on human blood agar	Hemolysis on sheep blood agar: Alpha	Beta	Cold	Hemolysis on rabbit blood agar	Coagulase production on human plasma	Coagulase production on rabbit plasma
4866	T	W	-	-	+	+		+	+	+	+
	N	W	-	-	+		+	+	+	+	+
	A	W	-	+	+				-	-	+
	G	O	-	+	+		+	+	-	+	+
K 1/3	T	O	-	-	+				+	+	+
	N	O	+	-	-				-	-	+
	A	W	-	-	-				-	-	-
	G	W	-	+	-				-	-	-
King	T	W	-	-	-				-	+	+
4955	T	W	-	-	+				-	+	-
K 2/4	N	W	-	-	+				-	+	-
	A	W	-	+	+				-	-	-
	G	W	-	-	+				-	+	+
5515	T										
	N	W	-	+	+		+	+	-	+	+
	A										
	G										
5190	T	O	-	+	+		+	+	+	-	-
	N										
	A										
	G	W	-	-	+			+	-	+	+
5071	T	W	-	-	+		+	+	-	+	+
	N										
	A										
	G										

Table 10. (cont.)

Culture number	Location	Pigment	Mannitol fermentation	Gelatin liquefaction	Hemolysis on human blood agar	Hemolysis on sheep blood agar: Alpha	Beta	Cold	Hemolysis on rabbit blood agar	Coagulase production on human plasma	Coagulase production on rabbit plasma
4991	T										
	N	W	-	-	-				-	-	-
	A										
	G										
5298	T										
	N	W	-	-	-				-	+	+
	A										
	G										
5439	T										
	N	W	-	+	+				+	+	+
	A										
	G										
5890	T										
	N	W	-	-	-				-	+	+
	A										
	G										
5938	T										
	N	O	+	+	+		+		+	+	+
	A	O	+	+	+	+		+	+	+	+
	G										
5731	T										
	N	W	-	-	+	+		+	-	+	-
	A	W	-	-	-				-	+	+
	G										
Cocker	T	W	-	+	+				-	+	+
	N	W	-	-	-				-	+	+

Table 10. (cont.)

Culture number	Location	Pigment	Mannitol fermentation	Gelatin liquefaction	Hemolysis on human blood agar	Hemolysis on sheep blood agar: Alpha	Beta	Cold	Hemolysis on rabbit blood agar	Coagulase production on human plasma	Coagulase production on rabbit plasma
5896	T	W	-	+	+				-	+	+
5379	N										
7457	T	W	-	+	-				-	+	+
	N	W	-	+	-				-	+	+
	A										
	G	W	-	+	-				-	+	+
7153	T	W	+	+	+		+		+	+	+
	N										
	A										
	G	W	-	-	+				-	+	+
6494	T										
	N	W	-	-	+				+	+	+
	A	W	-	-	-				-	+	-
	G	W	-	-	-				-	+	+
6782	T										
	N										
	A	W	-	-	+		+		-	-	-
	G	W	-	-	+		+		-	-	+
7279	T										
	N	W	-	-	-				-	+	+
	A	W	-	-	-				-	-	+
	G	W	-	-	-				-	+	+

Table 10. (concl.)

Culture number	Location	Pigment	Mannitol fermentation	Gelatin liquefaction	Hemolysis on human blood agar	Hemolysis on sheep blood agar: Alpha	Beta	Cold	Hemolysis on rabbit blood agar	Coagulase production on human plasma	Coagulase production on rabbit plasma
6514	T	W	-	-	+	+			-	+	+
	N	W	-	-	+				-	+	-
	A	W	-	-	-				-	+	-
	G	W	-	-	-				-	+	-
5071	T	W	-	-	+				+	+	+
	N	W	-	-	+				+	-	+
	A	W	-	-	-				-	+	-
	G										
7356	T										
	N	W	+	+	-				-	+	+
	A	W	-	-	-				-	+	+
	G	W	-	-	-				-	+	-
7369	T										
	N	W	-	-	+				-	-	+
	A										
	G	W	-	-	-				-	+	-
5636	T										
	N	O	+	+	+	+			+	+	+
	A	O	+	+	+	+			+	+	+

T = Throat
N = Nose
A = Axilla
G = Groin

Pigment Production

O = Orange
W = White

STUDIES ON THE STAPHYLOCOCCI OF CANINE ORIGIN WITH PARTICULAR REFERENCE TO THE CARRIER STATE OF PATHOGENIC STRAINS

by

P. SOUNDARA RAJULU

B. V. Sc., Madras University, 1940

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

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1959

The serious nature of the present day problem of staphylococcal disease in human beings is engaging the particular attention of the epidemiologists. In many of the advanced countries of the world investigators are regularly collecting complete data to maintain a surveillance to determine the presence of a problem, if one should exist, with a view to the immediate institution of control measures including the use of appropriate antibiotics. The National conference sponsored by the U. S. Public Health Service - Communicable Disease Center and the National Academy of Sciences (September 1958) had recommended among other things, that cultures should be made routinely for investigation, review and classification.

Realizing the importance of the carrier state in the transmission of a disease of this nature and the possibility of the existence of apparently harmless sources of infection, this investigation was undertaken to de - termine whether pet dogs acting as carriers, frequently play a role in such transmission among human beings.

The study comprised examination for the presence of staphylococci in the nose, throat and on skin of dogs. The sites selected on skin were (1) axilla and (2) groin. Canines visiting Dykstra Veterinary Hospital were utilized as sources of material. Staphylococcus medium 110 was used for the primary isolation of the organism and the strains isolated were subjected to the usual pathogenicity tests, viz., (a) coagulase production on citrated rabbit and human plasma, (b) production of hemolysis on 5 per cent human, rabbit and sheep blood agar plates. Pigment production, mannitol fermentation and gelatin liquefaction were also recorded. The coagulase positive strains were subjected to the action of human typing

phages, on the procedure recommended by the Communicable Disease Center and finally the typeable strains were tested for sensitivity to antibiotics.

Staphylococci were isolated from 30 (93.75%) of 32 dogs examined, from one or more situations. Twenty-eight dogs (87.5%) carried coagulase positive strains of staphylococci. The incidence of coagulase positive strains was found to be higher in the nose than in other locations.

Of the 65 strains examined for coagulase production, using both citrated human and rabbit plasma 49 strains (75.6%) coagulated human plasma and 46 strains (70.8%) coagulated rabbit plasma. The coagulase activity of the 65 strains was as below:

38 strains coagulated both human and rabbit plasma

11 strains coagulated human plasma alone

8 strains coagulated rabbit plasma.

Fifty-seven coagulase positive strains were subjected to the action of the basic set of human typing phages recommended by the International Sub-Committee on Staphylococcal Bacteriophage Typing, Stockholm, August 1958.

Nineteen of the 57 strains could be typed.

The above results show that these 19 strains can be identified as belonging to phage patterns characteristic of human strains. It is not possible at this stage to decide whether these strains with their phage patterns could be regarded as canine strains of the organism. It is reasonable to believe that these animals might have acquired their strains by close contact with human beings.

It is becoming increasingly evident that certain phage types of staphylococci are more or less characteristic of particular infections.

Allison (1949) and Allison et al. (1949) found that the staphylococci responsible for outbreaks of food poisoning were lysed by phages 6 and 47. Later work has shown that food poisoning staphylococcal strains virtually belong to the broad phage Group III (Anderson and Williams, 1956).

Of the 19 typeable strains studied in this investigation 14 were typeable wholly or predominantly by phages belonging to Group III, and phage 77 seemed to be the predominating single phage involved in 11 typings. Antibiotic resistance of these typeable strains was determined by growing each of these strains in the presence of antibiotic sensitivity discs. The following antibiotics were used:

(1) Penicillin (2) Dihydrostreptomycin

(3) Aureomycin (4) Neomycin (5) Erythromycin

(6) Bacitracin (7) Furadantin (8) Chloromycetin

(9) Kantrex (Kanamycin).

Out of these 14 strains typeable by phages of Group III, 12 showed remarkable resistance to broad spectrum antibiotics. This association of Group III strains with resistance to broad spectrum antibiotics had already been recognized in "Hospital Infections", i.e., epidemic strains (Anderson and Williams 1956; Blair and Carr 1958).

Taking all these observations into consideration, it may be believed that pet dogs were capable of harboring human epidemic strains of staphylococci with a certain possibility of their transmission among the members of their master's household.

Rountree et al. (1956) found six dogs harboring in their noses typeable strains of staphylococci.

7/42E/77	----	2
7/47D	----	1
3/B/44	----	2
42E/47	----	1

Coles (1958) did not find typeable strains among the strains of staphylococci isolated from canines and studied by him. Coles' observations combined with the present investigation strengthen the belief in the carrier state in canines. The species of bacteria isolated from the upper respiratory tract and skin vary from place to place and from time to time so that one observer may record a high incidence of particular bacterium, and another worker, other bacterial species.