

A SEROLOGICAL STUDY OF STREPTOCOCCI ISOLATED FROM FLUIDS AND TISSUES OF ANIMAL ORIGIN

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SUMMARY

A total of 668 strains of streptococci isolated from tissues and fluids of birds and animals was tested for haemolytic activity and reactivity with antisera for Lancefields Groups A-P and S.

It was not possible to group 362 strains. Distribution of strains in Lancefield groups was A, 1; B, 12; C, 101; D, 109; E, 1; F, 0; G, 3; H, 0; K, 8; L, 27; M, 0; N, 14; O, 8; P, 0; and S, 10. In addition, cross-reacting strains from pigs included GH, 1; HK, 8; GHK, 1; and GK, 2.

Attention is drawn to the occurrence of Group O strains in an outbreak of bovine mastitis and the predominance of Group D strains in those isolated from birds.

I. INTRODUCTION

With the advent of antibiotics active against streptococci, the identification and study of streptococci causing disease in domestic animals has been pursued less vigorously than formerly.

However, the identification of streptococci and the assessment of their pathogenicity is still a problem in a veterinary diagnostic laboratory for it is recognized that these bacteria are commonly found in the intestinal and respiratory tracts of normal animals and may persist for long periods in the animal environment.

Consequently, 668 strains of streptococci were examined to determine the number of strains in the various Lancefield groups.

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TABLE 1
LANCEFIELD GROUPS OF STREPTOCOCCI OF ANIMAL ORIGIN

—		No. Examined	Not Grouped	A	B	C	D	E	F	G	H	K	L	M	N	O	P	S	GH	HK	GHK	GK
Cattle	..	202	130	..	11	29	17	3	..	4	8
Goats	..	2	1	1
Horses	..	7	4	3
Mouse	..	1	1
Pigs	..	293	132	1	1	68	26	1	..	3	..	7	24	..	8	10	1	8	1	2
Rats	..	3	3
Seal	..	1	1
Sheep	..	33	27	3	1	2
Total	..	542	299	1	12	100	47	1	0	3	0	8	27	0	14	8	0	10	1	8	1	2

II. METHODS

Strains were isolated on 10 per cent. sheep blood agar from specimens submitted to the Animal Research Institute for bacteriological examination over the period 1953-1957. Although strains were collected as isolated, some selection of strains occurred. These selected strains included 30 strains isolated from normal and pneumonic pig lungs and 45 strains isolated from milk from cases of bovine mastitis and which were considered to be unusual as determined by colonial morphology, haemolytic activity or results obtained when tested according to the method of Slavin (1948).

The strains were sown onto blood agar slopes directly from the plate on which they were isolated and held on the slope until tested for haemolytic action and serological group.

Antisera for each of the Lancefield Groups A-P and S were prepared in rabbits (McLean 1955) and all strains were tested against these antisera using the methods of Lancefield (1933) and Fuller (1938).

Haemolysis was investigated on agar plates containing 10 per cent. horse blood incubated aerobically and anaerobically at 37°C for 24 and 48 hr (McLean 1955).

III. RESULTS

Tables 1 and 2 give details of the species of animals and birds from which the strains were isolated, the number belonging to each Lancefield group and those that could not be placed in any of the groups.

TABLE 2
LANCEFIELD GROUPS OF STREPTOCOCCI OF AVIAN ORIGIN

—	No. Examined	Not Grouped	Lancefield Groups	
			C	D
Budgerigar	5	1	1	3
Canary	2	2
Duck	6	5	..	1
Fowl	104	47	..	57
Galah	2	2
Goose	1	1
Parrot	3	3
Turkey	3	2	..	1
Total	126	63	1	62

Of the animal strains, those from goats, horses, rats, a mouse and a seal were too few to warrant comment. The cattle, pig and sheep strains will be considered in more detail with respect to tissue origin, haemolytic activity and aspects of special interest.

(i) *Cattle Strains*.—Table 3 gives the tissue or fluid from which the 202 strains were isolated. Of the 130 strains not grouped, 32 were non-haemolytic, 84 alpha-haemolytic and 14 beta-haemolytic.

TABLE 3
SOURCE OF STREPTOCOCCI ISOLATED FROM CATTLE

Tissue	Not Grouped	Lancefield Group					
		B	C	D	L	N	O
Abomasum	2
Abscess	1	..	1
Brain	1
Foetus	1
Heart	5	3
Intestine	1
Kidney	7	4	1	1	..
Larynx	1
Liver	21	..	1	3	..	1	..
Lung	14
Middle ear	1
Milk	44	11	26	3	..	2	8
Mesenteric lymph node ..	1	1
Muscle	3	2
Pericardium	4
Placenta	1
Prepuce	1
Retropharyngeal lymph node	1
Spleen	6	1
Thoracic lymph node ..	2
Trachea	1
Urine	2
Vagina	8	..	1	1
Whey	2
Total	130	11	29	17	3	4	8

The Group B strains were *Str. agalactiae*; 7 were non-haemolytic or alpha-haemolytic and 3 strains showed hazy beta-haemolysis. Twenty-six of the 29 Group C strains were isolated from milk and of these 8 were non-haemolytic, 3 alpha-haemolytic and 15 beta-haemolytic. The abscess strains were non-haemolytic and the vaginal and liver strains alpha-haemolytic.

The Group D strains were isolated from a variety of tissues and included 7 non-haemolytic and 10 alpha-haemolytic strains.

The kidney Group L strain was alpha-haemolytic and the two muscle strains, both from the same animal, were beta-haemolytic.

In the Group N strains, one milk strain and the liver strain were non-haemolytic and the other milk strain and kidney strain were alpha-haemolytic.

The Group O strains were from milk samples from one herd in which an outbreak of mastitis was occurring.

(ii) *Pig Strains*.—Of the 293 strains of porcine origin, 132 could not be grouped (Table 7). The distribution of haemolytic types within the non-grouped strains was non-haemolytic 19, alpha-haemolytic 108 and beta-haemolytic 5. Of the beta-haemolytic strains isolated, 4 were isolated from lung tissue and 1 from vaginal exudate.

The Group A strain was beta-haemolytic. The Group B strain was alpha-haemolytic and the grouping extract reacted weakly. Group C strains were most numerous and they were isolated from a wide variety of tissues. The organism was quite frequently isolated from cases of arthritis in young pigs. Strains of Group D were also frequently isolated.

Group E was represented by one beta-haemolytic strain, from heart tissue.

Two of the Group G strains isolated from the pericardial sac and lung of one pig were non-haemolytic and the third strain isolated from lung tissue was alpha-haemolytic.

Of the 7 Group K strains, 2 were non-haemolytic and 5 alpha-haemolytic. Three of the extracts reacted only weakly with the Group K antiserum. Of 24 strains of Group L isolated, 19 were from lung tissue. Seven of the strains were alpha-haemolytic and 17 beta-haemolytic.

Four of the 8 strains of Group N were isolated from the heart and pericardium. Four strains were non-haemolytic and 4 were alpha-haemolytic.

Twelve strains isolated from lung tissue all reacted with two or more antisera of Groups G, H and K. The GH strain was alpha-haemolytic, as also were the GK strains. The HK strains contained 6 non-haemolytic and 2 alpha-haemolytic. The GHK strain was beta-haemolytic.

Four of the Group S strains were alpha-haemolytic and 6 were beta-haemolytic. The high number of isolations reported from joints in Table 4 is without significance in regard to the frequency of occurrence of this organism in pigs, as more than one joint of a piglet may be cultured.

(iii) *Sheep Strains*.—Of the 33 strains from sheep, 27 were not grouped. The sources of these 33 strains are given in Table 5. No beta-haemolytic streptococci were isolated. Eight, including the three Group D strains, were non-haemolytic and the remaining 25 were alpha-haemolytic.

TABLE 4
SOURCE OF STREPTOCOCCI FROM PIGS

Tissue	Not Grouped	A	B	C	D	E	G	K	L	N	GH	HK	GHK	GK	S
Abscess	1
Brain	1	1	1
Heart and pericardium	16	5	3	1	1	1	..	4
Intestine	2
Joint	4	14	1	2	6
Kidney	15	7	2
Liver	19	13	5	3	1	1
Lung	53	1	1	16	6	..	2	2	19	2	1	8	1	2	1
Mesenteric lymph node	4
Milk	1	1
Muscle	1
Pleural fluid	1	2
Peritoneal fluid	3	1
Semen	2	2
Spleen	3	2	2	1	1
Stomach	1
Throat	6
Uterus	1
Vagina	4	6	3
Total	132	1	1	68	26	1	3	7	24	8	1	8	1	2	10

TABLE 5

SOURCE OF STREPTOCOCCI FROM SHEEP

Tissue	Not Grouped	D	K	N
Abomasum	2
Heart	4
Joint	6	2
Kidney	1	1
Mesenteric lymph node ..	2
Liver	5	1	..	1
Lung	3
Prepuce	1
Spleen	1
Thoracic fluid	1
Uterus	1
Vagina	1	..
Total	27	3	1	2

TABLE 6

SOURCE OF STREPTOCOCCI FROM FOWLS

Tissue	Not Grouped	Group D
Abscess	1
Air sac	1
Brain	2
Eye	2	3
Heart	5	4
Intestine	1	..
Joint	4	12
Liver	14	16
Lung	10	15
Pericardial sac	1
Sinus	2	1
Spleen	1	..
Trachea	6	1
Umbilicus	1	..
Viscera	1	..
Total	47	57

(iv) *Avian Strains*.—These were noteworthy in that only one Group C and 62 Group D strains were detected in 126 cultures tested (Table 2). Fowl strains were either not groupable or belonged to Group D.

IV. DISCUSSION

As selection was practised to some extent in the choice of strains for testing, the results cannot be considered as reflecting the distribution of Lancefield types in animals and birds in the specimens submitted. However, it is noteworthy that approximately 54 per cent. of the cultures, some of which were beta-haemolytic, could not be placed in any of the 14 Lancefield A-P and S groups.

Of the strains isolated from cattle, the Group O strains are of special interest. This group was delineated in 1950 by Boissard and Wormald (1950), who studied the biochemical characteristics of 34 strains and reported that they had identified over 90 such strains from swabs of healthy human throats. To our knowledge the only other report of streptococci of O group is that of Farmer (1953), who isolated seven strains from normal human mouths. The Group C bovine strains included some identified as *Str. zooepidemicus* and these were considered to be responsible for an outbreak of mastitis.

None of the three Group L cattle strains were isolated from milk, although Olsen (1956) has reported it as a cause of bovine mastitis and suggested that the source of the strains could be pigs or man. The reported isolation in this paper of 24 strains of Group L from pigs, including 19 from lungs, would support the view of Olsen (1956). The physiological characteristics of 16 of the 24 porcine lung Group L strains have been described by McLean (1955). The pig may be considered as a host for streptococci of many groups. Collier (1951) carried out a serological examination of 67 strains of beta-haemolytic streptococci from pigs and reported that 43 strains belonged to Group C, 13 to Group L, 5 to Group E and 6 could not be classified into any of the Lancefield Groups A-P.

Collier's strains were all from pathological lesions and therefore not comparable with those reported in this paper. It is of interest, however, that 5 non-grouped beta-haemolytic strains were found in the 293 strains of porcine origin in our work. Collier (1954, 1956) has described streptococcal lymphadenitis of the pharyngeal region of pigs caused by Group E streptococci. This syndrome has not been seen in pigs in Queensland and the single Group E strain isolated was not associated with such a condition.

It is of interest that four of the eight Group N strains in pigs were from heart tissue, as in at least one of the cases from which it was isolated it was thought to be the cause of a vegetative lesion in the heart. As mentioned above, Group L occur commonly in pigs and where pigs and cows are kept together the organism may spread from pigs to cows, resulting in mastitis (Olsen 1956).

Besides the original account (Field, Buntain, and Done 1954) the isolation of streptococci of Group S has been recorded in New Zealand (Anon. 1956, p. 29), although in this instance the streptococci appear to have been checked only biochemically and not serologically, and the Netherlands (de Moor 1959). In Queensland and New Zealand, the arthritis described by Field, Buntain, and Done (1954) in England has been seen in the cases presented for examination.

The New Zealand workers reproduced arthritis in two pigs with the strain which they had isolated but could not produce lesions in the brain or meninges similar to those described by Field, Buntain, and Done, though there were some symptoms attributable to central nervous dysfunction.

Streptococci belonging to Group C have been isolated also from piglets showing lameness and nervous disorders at this Institute. Group C streptococci were isolated from milk from a case of porcine mastitis during the survey, and subsequent isolations made at this Institute would suggest that suckling piglets could quite easily contract infection from this source.

The occurrence of non-haemolytic Group G is unusual, as both large and minute colonial forms are said to be beta-haemolytic (Wilson and Miles 1955).

In the pig strains, some reacted, although weakly, with two or more antisera of Groups G, H and K. The significance of this cross-reaction is not known.

The avian strains gave somewhat unexpected results because of the preponderance of Group D strains. It is believed that these strains may be responsible for septicaemia in baby chicks as a result of infection in the incubator or brooder, and that in some instances the organism may localize in joints to produce arthritis. Whether any specific strains of Group D streptococci have special pathogenicity in this regard is not known.

The large number of non-grouped strains isolated indicates the amount of work required to obtain a better knowledge of their identity and ecology.

V. ACKNOWLEDGEMENTS

I am indebted to Mrs. S. J. May (Perth, Western Australia) who carried out the grouping of the streptococci and to officers of this Institute who isolated strains of streptococci reported on this paper.

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(Received for publication March 8, 1963)