BACTERIOLOGICAL STUDIES ON STREPTOCOCCI FROM BOVINE UDDER II.

DISTRIBUTION OF *STR. AGALACTIAE* IN DOMESTIC ANIMALS AND LONG-TERM OBSERVATIONS ON THE UDDER WHICH HARBOURED THE ORGANISM

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PREFACE

It may be probable that the natural habitat or reservoir of *Str. agalactiae* is the bovine udder. The detections of this organism from cows from parts other than their udders or milk samples seem to be rare. HARRISON reported negative results on vaginal swabs of 50 cows and 20 virgin heifers, and on dung of 24 cows which were suffering from clinical mastitis. FRANCIS also made a bacteriological examination of 100 pairs of bovine tonsils and 40 vaginas, but he failed to isolate this organism. Following "Bovine Mastitis" edited by LITTLE & PLASTRIDGE, at the Storrs Experiment Station, over 300 specimens of the body fluid or tissues from sick or dead cattle (excluding udders and milk samples) have been examined bacteriologically. In no instance have streptococci which were identified as agalactiae been isolated. However, BULL et al in Australia reported the isolation of 8 strains of this organism among 224 streptococci from feces. Recently FORNONI in Italy described the isolation of *Str. agatactiae* from the bovine tonsils in 8 among 222 samples.

Anyhow, it may be said that the rate of occurrence of *Str. agalactiae* in cows tissue other than the udder is pretty low, and seems to have not so much responsibility for other infections than bovine mastitis. Reports concerning them are easily countable, namely lung abscess of dairy calf by DHANDA & SINGH, multiple cutaneous abscess in captured elephant by ST. GEORGE & PILLAI, abortion of horse, swine and rabbit or endometritis of cow by EDWARDS, and

several interesting cases in human beings summarized in the textbook "Biologie der Streptokokken" by M. SEELEMANN, etc.

From the above data, it is probable that the detection of *Str. agalactiae* in the bovine tissues or secretion other than the udder and milk samples is very rare, however as to the presence of this organisms in domestic animals except the cow, it has been scarcely studied. Also there seem to be some workers who are wondering about the pathogenicity of this organism because it is often demonstrated from the normal bovine milk.

In these circumstances, it is very interesting and valuable to investigate these problems. The data obtained by the present authors are described in this report.

MATERIALS AND METHODS

A. Studies on the distribution of *Str. agalactiae*

1. Strains obtained

The organ materials were aseptically collected from animals which had been condemned at the Sapporo abattoir. The materials examined are as follows: cattle (108 tonsils, 104 small intestinal contents, 102 caecal contents, 100 rectal contents, 95 uterine mucus, 106 nasal fluid, 94 teat skin, 95 vaginal mucus, 99 urines and 291 milk samples), horses (105 nasal fluid, 112 tonsils, 100 caecal contents, 18 rectal contents and 50 urines), swine (101 tonsils, 63 caecal contents and 21 vaginal mucus) and sheep (66 tonsils, 59 caecal contents, 42 nasal fluid and 11 uterine mucus).

The cultures were made within 24 hours after death or sampling. The surface of the tonsils was sterilized by cotton soaked in disinfectant alcohol and by using sterilized wire needles, the samples from inside of lacunae were obtained and cultivated. Usually 5% sheep blood agar plate was employed, in some cases EDWARDS' esculin crystal violet blood agar, and the materials were directly inoculated on them. After 48 hours at 37°C, the streptococcal colonies resembling agalactiae were picked out and submitted to the further examinations. These samples numbered 723.

2. Identification of strains

The streptococcal strains which showed the next described biochemical features were suspected to be *Str. agalactiae*: hydrolysis of sodium hippurate, non-hydrolysis of esculin, production of ammonia from arginine, and non-fermentation of mannitol, sorbitol, inulin and raffinose. As to the strains presenting the above characteristics, the precipitation reactions following LANCEFIELD which are precisely reported in the senior author's previous report, were tested and the strains reacting with group-B sera were finally determined definitely as *Str. agalactiae*.

B. Studies based on the long-term observations on cows which harboured *Str. agalactiae*

One loopful respectively of aseptically drawn milk samples was directly cultivated on 5% sheep blood agar. When the direct cultivations of the milk samples proved sterile and

Hotis test positive, additional cultures were made from Hotis test tubes. As to the agalactiae-like colonies, the above described methods were adopted for their identification. The other organisms were determined chiefly by following methods described in "Bergeys' Manual of Determinative Bacteriology". Several mastitis tests such as cell count, B.T.B. test, etc. were performed following "Bovine Mastitis" edited by LITTLE & PLASTRIDGE.

RESULTS

1. Distribution of *Str. agalactiae* in domestic animals

Among the 1942 samples from 4 species of domestic animals, total 723 streptococcal strains which showed agalactiae-like growth, were selected as are indicated in table 1. Afterwards, they were submitted to examination for certain biochemical characteristics

TABLE 1. *No. of Materials and Source of Strains Examined*

NUMBER OF STRAIN EXAMINED BIOCHEMICALLY	Animal Species	SOURCE	
		Kinds of Materials	No. of Samples Examined
49	Cows	Tonsil	108
36		Small intestinal contents	104
15		Vaginal mucus	95
59		Nasal fluid	106
42		Teat skin	94
12		Urine	99
36		Rectal contents	100
10		Uterine mucus	95
37		Caecal contents	102
85		Milk	291
39	Horses	Nasal fluid	105
42		Tonsil	112
85		Caecal contents	100
2		Urine	50
6		Rectal contents	18
71	Swine	Tonsil	101
12		Caecal contents	63
0		Vaginal mucus	21
50	Sheep	Tonsil	66
26		Caecal contents	59
9		Nasal fluid	42
0		Uterine mucus	11
Total 723			1942

TABLE 2. *Results of Precipitation Reactions on the Strains which showed some Biochemical Features Resembling Str. agalactiae or Str. dysgalactiae*

STRAIN			NO. OF STRAIN SEROLOGICALLY TESTED	RESULTS OF PRECIPITATION
Showing	No.	Source		
	1	Cow nasal fluid	1 ·····	1*
	1	Cow uterine mucus	1 ·····	Group-A
	9	Cow milk	9 ·····	2···Group-B, type II 5···Group-B, no polysaccharide type 2*
HEA +−+				
MANRISO −	4	Horse nasal fluid	3 ·····	1···Group-B, no polysaccharide type 2*
	1	Horse tonsils	1 ·····	Group-B, type I
	6	Swine tonsils	4 ·····	1···Group-B, no polysaccharide type 2···Group-C 1*
	2	Cow tonsils	1 ·····	Group-C
	2	Cow small intestinal contents	2 ·····	1···Group-D 1*
	2	Cow nasal fluid	1 ·····	Group-D
	1	Cow uterine mucus	0	
	2	Cow caecal contents	0	
HEA −−+	4	Cow milk	3 ·····	2···Group-C 1*
MANRISO −	1	Horse nasal fluid	0	
	14	Horse tonsils	5 ·····	2···Group-C 3···Group-L
	1	Horse caecal contents	1 ·····	Group-D
	1	Horse rectal contents	1 ·····	Group-C
	13	Swine tonsils	6 ·····	1···Group-C 1···Group-B, no polysaccharide type 2···Group-D 2*

Remarks * Shows the strains which did not react with the sera of Groups A, B, C, D, E, F, G, H, K, L, M, N and O.

HEA +−+ means hydrolysis of sodium hippurate, non-hydrolysis of esculin and production of ammonia from arginine.

MANRISO − means non-fermentation of mannitol, raffinose, inulin and sorbitol.

All of Group-C organisms, which were serologically identified, showed β-hemolysis.

described above under "identification of strains". From those examinations it became clear that the number of strains showing some biochemical characteristics resembling those of *Str. agalactiae* (sodium hippurate +, esculin −, ammonia from arginine +, fermentation of mannitol, sorbitol, inulin and raffinose −) was 22 and of the strains resembling *Str. dysgalactiae* was 43.

The authors could not test the precipitation reactions of all these strains, because some of them showed poor growth on further cultivation and finally disappeared. These results are detailed in table 2.

The authors made serological examinations of 19 strains among 22 which showed some biochemical features resembling those of *Str. agalactiae* and detected 10 strains which reacted with group-B serum. By the same procedures with the above, the authors could also detect *Str. agalactiae* in 1 strain among the samples which showed some biochemical features resembling those of *Str. dysgalactiae*.

From these experiments, it may be said that the distribution of *Str. agalactiae* in domestic animal bodies is very rare, as is summarized in table 3; one from 105 horse nasal fluid (0.95%), 1 from 112 horse tonsils (0.89%) and 2 from 101 swine tonsils (1.97%), but, never from cows except from milk samples (7 among 291···2.4%).

TABLE 3. *Summarized Data of the Str. agalactiae Detected Cases in the Materials from the Sapporo Abattoir*

MATERIALS	NO. MATERIALS EXAMINED	NO. STRAINS EXAMINED	NO. *STR. AGALACTIAE* DETECTED	SEROTYPE OF *STR. AGALACTIAE*
Cow's milk	291	85	7 (2.4%)·····	Type II ····· 2 No polysaccharide type ····· 5
Horse nasal fluid	105	39	1 (0.95%) ·····	No polysaccharide type
Horse tonsil	112	42	1 (0.89%) ·····	Type I
Swine tonsil	101	71	2 (1.97%) ·····	No polysaccharide type

2. Distribution of *Str. agalactiae* in the cows milk near Sapporo

The survey was carried out in Ishikari District near Sapporo during the period from August to September, 1958.

The total number of cows examined was 217 head comprised of 855 quarters, the animals were fed by 78 owners.

In these milk samples, the detection of *Str. agalactiae* was 14.6% in mastitis or abnormal milk (59 quarters among 404) and 1.6% in normal milk (7 quarters among 451) which will be seen from table 4. The mean value of the detection rate in these cows was 7.7% (66 quarters among 855); this percentage is greater than that obtained from the milk samples from the Sapporo abattoir.

Concerning the serological type distribution of *Str. agalactiae* from the Hokkaido milk samples tested, the data are recorded in table 5. Type II is of most frequent occurrence among them with non-polysaccharide type and type III ranking next. The types Ia and

TABLE 4. *Detection of Str. agalactiae from Cow's Milk near Sapporo* (August 1958)

NO. OF COWS EXAMINED	NO. OF QUARTERS EXAMINED			DETECTED OF *STR. AGALACTIAE*
217	855	Mastitis and abnormal milk	404	59 (14.6%)
		Normal milk	451	7 (1.6%)

Remarks 1. Including the normal milk, streptococci were isolated in a total of 84 cows (38.7%) which comprised from 165 quarters (19.2%).
2. In this case, "abnormal milk" corresponds to that which shows higher than pH 6.6 by B.T.B. test or more than half million cell count.
3. Total 107 strains among 129 streptococci detected from abnormal or mastitis milk samples were identified and also 25 among 36 from normal milk samples.

TABLE 5. *Serotyping of Str. agalactiae from Bovine Milk in Hokkaido*

NO. OF STRAINS EXAMINED	RESULTS OF SERO-TYPING				
	Ia	Ib	II	III	No Polysaccharide
198	0	0	119 (60.1%)	25 (12.6%)	54 (27.3%)

Ib have never been detected from the bovine milk to the present in Hokkaido.

The serotypes of 4 agalactiae strains isolated from horse and swine are indicated in table 3; no polysaccharide type was detected more frequently, being 3 strains out of 4. Detection of type I from horse tonsil, which indicates strong positive reaction with both Ia and Ib type sera, was interesting because such serotype has never been detected from bovine milk in Hokkaido.

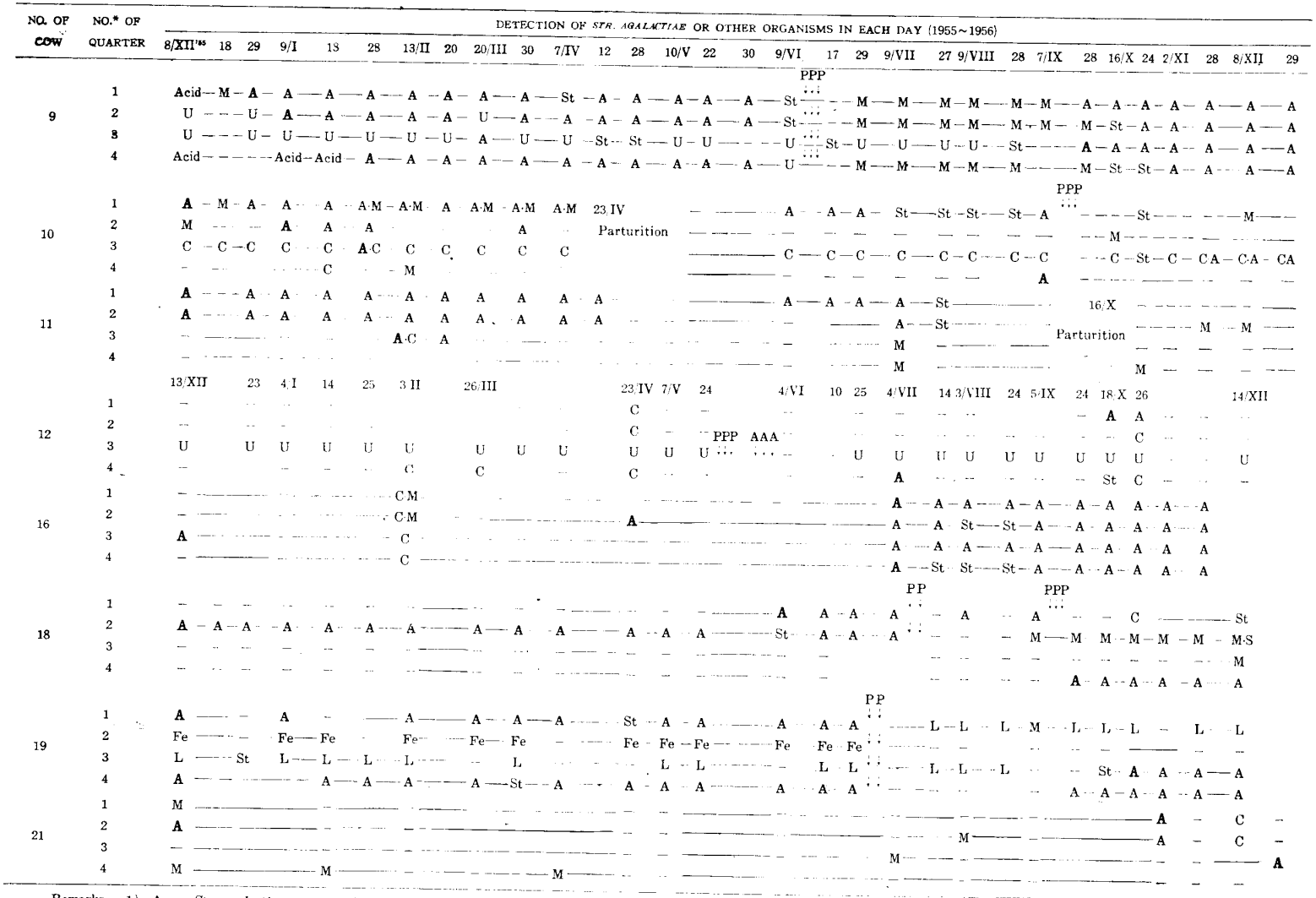
3. Long-term observations of cows which harboured *Str. agalactiae*

On the investigation of transmission of *Str. agalactiae* by milking with infected hands, LANCASTER & STUART have already reported very interesting results. The next described data were observed by the present authors in natural conditions in Hokkaido.

In 1 herd which comprised from 30 cows, in bovine mastitis of type II of group-B streptococci heavily prevailed, bacteriological observations were carried out at about 10 day intervals during the period from December 8th, 1955 to December 28th, 1956. As to the spreading of *Str. agalactiae* from one quarter to another, some interesting cases are indicated in chart 1.

Gradual spread of quarter infections of *Str. agalactiae* will be easily seen from the chart, especially in cases No. 9, No. 10 and No. 18. Infections due to organisms other than *Str. agalactiae* showed the tendency to be replaced by *Str. agalactiae* as in case No. 9.

CHART 1. *Spreading of Str. agalactiae in the Udders of the Infected Cow*



Remarks 1) A ····· *Str. agalactiae*
U ····· *Str. uberis.*
Acid ··· *Str. acidominimus.*
Fe····· *Str. faecalis.*
L ····· *Str. lactis.*
S·····Streptococci other than *Str. agalactiae.*
St·····Not identified streptococci.
M·····Micrococci.
C·····Corynebacterium.

2) * 1·····left front.
2·····right front.
3·····left rear.
4·····right rear.

3) Arrows indicate 200,000 units of penicillin (P) or 426 mg of aureomycin ointment (A) injection daily per quarter.

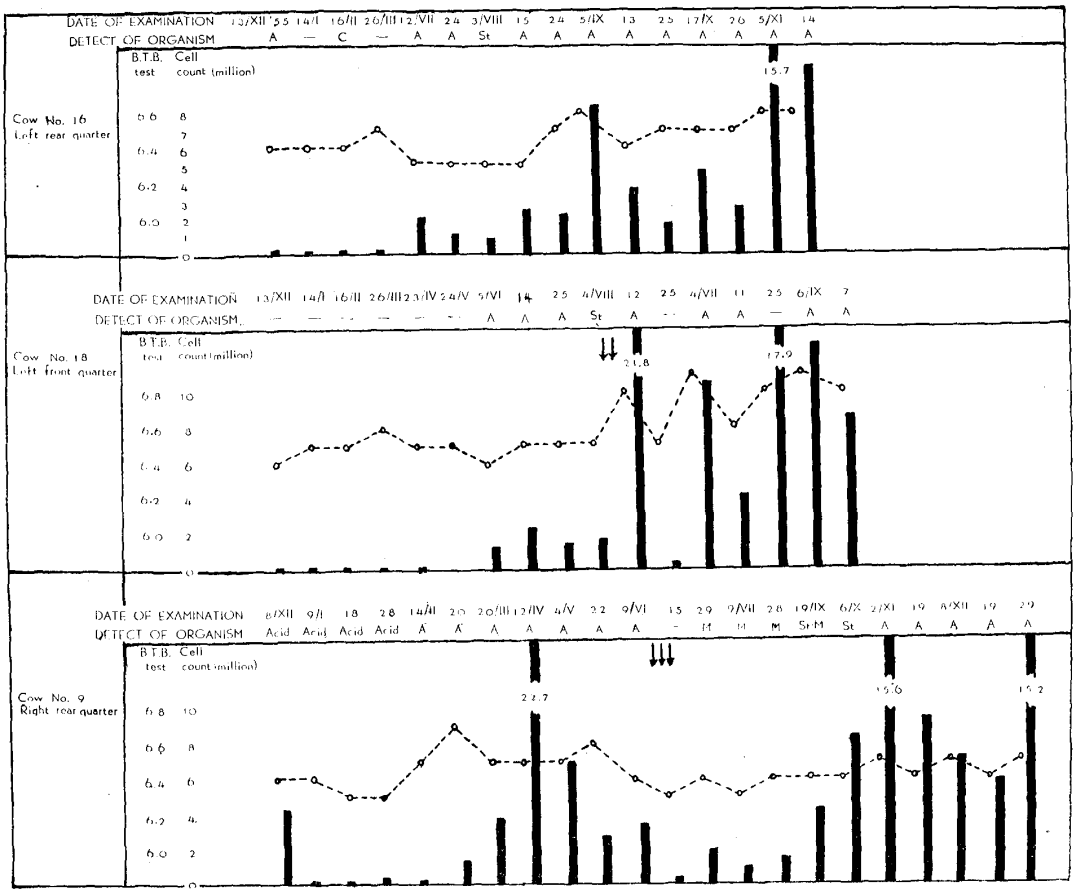
Str. agalactiae was detected from 14 cows among 30 (46.7%) during the observation period.

Such change of bacterial flora in each quarter may be caused by many factors; spontaneous ones such as parturition in cases No. 10 and No. 11, or artificial factors such as therapeutical treatment as is in cases No. 9, No. 18 and No. 19.

In some cases such as in quarter No. 2. of cases No. 10 and No. 11, spontaneous disappearance of *Str. agalactiae* from milk had occurred after the cow had calved. The injection of penicillin or aureomycin into the udder often caused temporary disappearance of *Str. agalactiae* or replacement of the bacterial flora by micrococci or other bacteria. But soon after this, in accordance with the weakening of the therapeutical effect, *Str. agalactiae* will sometimes appear again and the infection will persist for long although of course, complete recovery sometimes occurs. The above stated facts can be read from chart 1.

The quarter which is harbouring *Str. agalactiae* usually shows mastitis or milk

CHART 2. *Changes in Milk Qualities following the Appearance of Str. agalactiae*

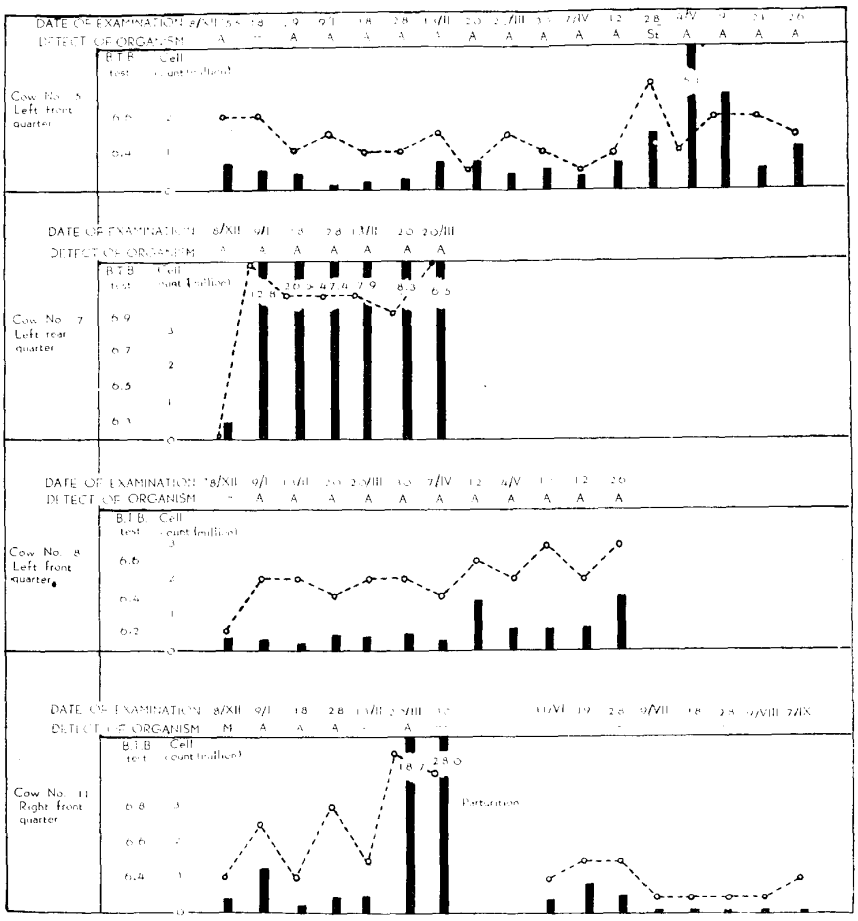


Remarks A……*Str. agalactiae.*
St……Streptococci, not identified.
Acid…*Str. acidominimus.*
C……Corynebacterium.
M……Micrococci.

Arrows indicate the injection of 200,000 units of penicillin daily per quarter.
▮ …Cell count.
○---○ …B.T.B. test. (pH value)

abnormalities such as increased cell counts, positive Whiteside test, or B.T.B. test, etc. As previously stated, the authors sometimes observed that several quarters secrete normal milk regardless of harbouring *Str. agalactiae*. From these findings, one sometimes may be likely to regard *Str. agalactiae* as just like a non-pathogenic organism to imagine an extreme case. One, however, must remember that these findings were usually derived from a single examination on the same cow. The results of long-term observations on the same cow, which secreted normal milk in spite of harbouring *Str. agalactiae* will be seen in charts 2 and 3. All cases developed until they presented mild or severe milk abnormalities or mastitis in the course of these observations though at first the milk showed normal.

CHART 3. *Changes in Milk Qualities following the Appearance of Str. agalactiae*



Remarks Signs employed in this chart are those in chart 2.

Usually milk abnormalities occur within a short time after the invasion of the organisms as is in cow No. 7 in chart 3, sometimes in 2~3 months as is in cow No. 11 in chart 3 and cows No. 9, No. 16 and No. 18 in chart 2. There are some cases which did not indicate marked increase of cell counts through a considerably long period had elapsed as in the

cases of cows No. 5 and No. 8 in chart 3.

On the pathogenicity of *Str. agalactiae*, it is natural to say that the susceptibility of the cow is the most important factor. Anyhow, the authors are confident that the presence of *Str. agalactiae* in the bovine udder may be considerably dangerous for the cow's future development of milk abnormalities or mastitis, even if the animal remains normal for a short time.

DISCUSSION

From the present studies on the distribution of *Str. agalactiae*, the authors also could recognize that this organism has a very intimate relationship with the cow, being limited only to their udder. In spite of examining organ materials from more than 100 head, the authors failed to find out this organism in the cow bodies except in their milk.

The present results are not coincident with the data on cow presented by FORNONI in Italy (8/222···3.6%) but with those of the Storrs Experiment Station or those of FRANCIS. This may not be due to the difference of technics or culture media employed, because the same organism could be isolated from swine tonsil in 1.91% (2/101).

From the data previously reported by the senior author and the present report, streptococcal mastitis does prevail among cows in Hokkaido, however, detection of *Str. agalactiae* is generally not so high on the average (about 15～20%) though the percentages diverge widely by herd or by district. These facts may exert some influence on the chance of detection from bovine tonsil.

As to the serotype of *Str. agalactiae* detected from sources other than the bovine milk, it is difficult to offer discussion because of the small number of strains. However, isolation of type I organism from the horse tonsil was somewhat interesting, because this type has never been detected from the bovine milk in Hokkaido, nor in England following the report by PATTISON et al., regardless of the surveys on a considerably large number of the strains.

In case of the agalactiae infection, it is generally accepted that the susceptibility of the cow is important. The cows which have non-susceptibility, are resistant to the invasion of this organism. However, from the present observations it has become clear that the cows which were once invaded by this organism usually developed until they manifested the milk abnormalities or mastitis during the long observation period even if their milk showed normal at that time. Accordingly, it may be said that detection of this organism in a cow's milk samples should be regarded as a danger warning for future manifestation of disease.

SUMMARY

Bacteriological studies were carried out on the distribution of *Str. agalactiae* in organ materials of domestic animals, which were obtained from the Sapporo abattoir, including cows milk from herds near Sapporo. Long-term observations on the cows which harboured *Str. agalactiae* in their milk samples were also made. The data obtained are summarized as follows:

1) Streptococcal strains to the number of 723 which were obtained from samples from 4 species of domestic animals (horses, sheep, cows and swine) were biochemically examined (table 1).

2) Among 65 strains which showed some biochemical characteristics resembling those of *Str. agalactiae* or *Str. dysgalactiae*, 39 strains were serologically diagnosed (table 2).

3) *Str. agalactiae* was detected from 4 materials other than cow's milk: 1 from horse nasal fluid (1/105···0.95%), 1 from horse tonsils (1/112···0.89%) and 2 from swine tonsils (2/101···1.9%) respectively (table 3).

4) Detection of *Str. agalactiae* from cow's milk tested near Sapporo was 14.6% in mastitis or abnormal milk (59/404) and 1.6% in normal milk (7/451). The survery was conducted on 217 cows which were fed by 78 owners (table 4).

5) From the above facts, it will be seen that *Str. agalactiae* has a very intimate affinity for the cow's udder.

6) *Str. agalactiae* showed the tendency to expel the infections caused by other organisms and gradually to invade from one quarter to another (chart 1).

7) The cows which harbour *Str. agalactiae* in their milk will usually show mastitis or various degrees of milk abnormalities before long, even if their milk showed normal for the time being (charts 2 and 3).

8) The authors believe that the presence of *Str. agalactiae* in a cow's milk may result in the development of mastitis sooner or later.

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