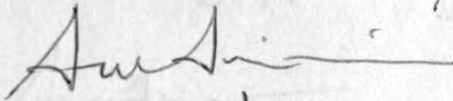THE OCCURRENCE OF STREPTOCOCCUS AGALACTIAE IN
BULK MILK OF SELECTED DAIRY HERDS IN KENYA

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A thesis submitted in part fulfilment for the Degree
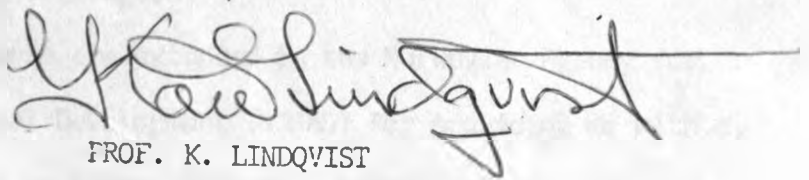of Master of Science in the University of Nairobi.

1979

DECLARATION

This thesis is my original work and has not been presented
for a degree in any other University.

S. MUTWIRI ARIMI

This thesis has been submitted for examination with my
approval as the University Supervisor.

PROF. K. LINDQVIST

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ABSTRACT

The purpose of this study was to screen bulk milk for the occurrence and distribution of Str. agalactiae in selected dairy herds in Kenya. The areas of study were:- (a) Mt. Kenya region (b) Nairobi area (c) The Rift Valley Province and the adjacent areas of Central Province. The period of study covered April 1977 to July 1978.

Milk samples were collected from Kenya Co-operative Creameries where farmers sell their milk. Samples of 10 to 20 ml were taken from the delivery cans at the time of arrival at the creameries. The following investigations were carried out on the samples: (1) The California Mastitis Test (CMT) (2) examination for the presence of bacterial inhibitory substances and (3) examination for the presence of Str. agalactiae.

From the Rift Valley Province and the adjacent district of Nyandarua, Central Province, 906 samples were examined. Of these, 226 (24.9%) were CMT positive (≥ 3), 370 (40.8%) CMT negative (≤ 2) and 310 (34.2%) clotted or sour.

Sixty five samples (7.2%) were positive for bacterial inhibitory substances before heating. After heating for 5 minutes at 80°C, 24 of the 65 samples were positive for nonnatural inhibitors. Of these, 7 contained penicillin and 17 other unidentified inhibitors.

From Mt. Kenya region and Nairobi area, 352 samples were examined using Edwards medium (modified) and Lancefield's method of grouping. No Str. agalactiae was isolated.

Following the failure to isolate Str. agalactiae using
the modified Edwards medium, thallium-crystal violet-toxin
(TKT)-medium (modified Edwards medium containing 1.8%
staphylococcal β-toxin and 7-8% washed bovine erythrocytes)
was adapted to examine the 906 samples utilising the CAMP
reaction as the primary identifying characteristic. It was found
that β-toxin heated to 92°C and preserved at 4°C retained its
activity for more than a year. One to two per cent of the β-toxin
gave the best CAMP reaction and 1.8% was therefore used
throughout the investigation. CAMP positive / esculin negative
reactions were found to be good criteria for presumptive
identification of Str. agalactiae.

Twelve samples (1.3%) out of 906 yielded Str. agalactiae.
All the 12 isolates were confirmed to be group B by the slide
co-agglutination method of Christensen et al.(1973). Eight of
these isolates were from samples negative in the CMT. The
organism was isolated from 3 samples positive for bacterial
inhibitory substances other than penicillin.

The co-agglutination method was found to be cheap, rapid
and reliable. The use of 0.1 ml of antiserum to coat two or
more batches of staphylococci saves a lot of the expensive
antiserum. This makes the method of co-agglutination highly
economical.

Trypsinized bacterial sediments of two isolates, both
from Eldoret exhibited auto-agglutination. However, supernate.
of cultures of these strains reacted specifically with the
anti-group B co-agglutinating reagent. The use of culture

supernates from the few isolates examined in this study
was found to be reliable. Supernates of all the 12 isolates
agglutinated well with anti-group B co-agglutinating reagent.

Recovery of Str. agalactiae from bulk milk on a single
occasion is considered a reliable indication of mastitis in
a herd or the presence of healthy carriers which represent a
source of infection. However, a large proportion of the samples
were heavily contaminated with mainly esculin splitting group
D streptococci. A total count of >5.74 x 10^9 bacteria per ml
of bulk milk was recorded for 5 samples collected from KCC
Nakuru. Of these, 72.1% were esculin splitters and 27.4% non-
esculin splitters. With such heavy contamination, the TKT-
medium was found to be severely discoloured. Undiluted milk
causes a cofusing darkening of the medium (Munch-Petersen,
1947). Further tests of the method showed that CAMP reaction
is difficult or impossible to read under such conditions.
Therefore, an unknown number of Str. agalactiae positive
samples might have passed undetected.

An attempt was therefore made to improve the sensitivity
of the TKT-medium, using maltose and heated milk whey. The
sizes of the CAMP reaction zones were substantially increased
by the incorporation in the TKT-medium of maltose or milk
heated to 80°C for 2 minutes. The heated milk however,
imparted an undesirable cloudy (milky) background appearance
to the medium. The whey of the heated milk was found to
contain the substance(s) enhancing the CAMP reaction and to
provide a clear background. No difference in effect was
found between whey produced by acid or rennin coagulation

of milk. Rennin coagulation was found to be the most convenient.

The sizes of the CAMP reaction zones increased with increasing maltose concentrations up to about 1% (w/v). The sizes of the zones decreased with increasing maltose concentrations above 1%. Concentrations between 0.5 and 1% gave the largest CAMP reaction zones. In one experiment, 0.5% and 1% maltose gave average zone diameters of 3.9 and 3.8 mm respectively as compared with the TKT-medium control (2.0 mm). In another experiment, 0.5% and 1% maltose gave average zone diameters of 2.2 mm and 2.8 mm respectively as compared with the control (1.1 mm).

Although the incorporation of maltose and / or whey into the TKT-medium would be of great advantage, it was observed that in our samples of heavily contaminated milk, the addition of maltose, heated milk or whey does not improve the readability of the CAMP reaction. However, the increase in sizes of the CAMP reaction zones by the addition of maltose, whey or a combination of both, would be of distinct advantage in areas where the use of the TKT-medium is not impaired by heavy contamination of the bulk milk samples. Under conditions of low contamination, the larger CAMP reaction zones obtained by the medium developed in the course of this investigation will facilitate the detection of CAMP positive organisms, since the β-hemolytic zones of CAMP negative organisms do not show any increase in size in this medium.

INTRODUCTION

In dairy farming, bovine mastitis is a well documented
cause of severe losses in milk production all over the world
(Dobbins, 1977; Hughes, 1953; Little et al., 1946a; Mcleod
and Wilson, 1951; Stableforth, 1959; Weitz, 1971).

Among the rather limited number of causitive organisms,
Streptococcus agalactiae (Str. agalactiae) occupies a unique
position. It is the main cause of chronic and subclinical mastitis
which in its incipient course may go undetected for long
periods of time with subsequent decreased milk production which
may continue unnoticed by the farmer. Str. agalactiae has an
established pathogenic effect on the bovine mammary gland, and
because of this specificity, it is rarely found in other
pathological conditions in animals or man. There are however,
a few exceptions. Furthermore, Str. agalactiae has been highly
susceptible to penicillin and this property has not changed in
spite of the extensive use and abuse of this drug (Schalm, 1977).
By utilising these two characteristic features, udder specificity
and susceptibility to penicillin, the complete elimination of
Str. agalactiae from a dairy herd appears to be feasible
(Newbould, 1975). It is clear that a degree of contamination
of the environment must necessarily occur, but the survival
period of Str. agalactiae in the extramammary environment is
short, usually 3 weeks (Harrison, 1941; Philpot, 1975).
Therefore, hygienic measures must be included to provide an
effective eradication programme, both to eliminate the organism

from the contaminated areas in order to prevent reinfection from sources within the herd as well as from outside (Dobbins, 1970; Dodd, 1971; Kirkbride, 1975; Blood, 1978).

Clinical cases of mastitis have been recognized as a significant dairy problem in Kenya (Kariuki, 1978; Ward, 1978), which shows the need for the initiation of mastitis control/ eradication programmes. Some information about the disease is necessary as a prerequisite to such programmes. Very few studies have been done on this disease in this country. The scanty information available (Lauerman et al., 1973; Kenya Government Veterinary Annual Reports, 1950-71; Kariuki, 1978) show a very low recovery of Str. agalactiae from mastitis milk in some areas and a fairly high recovery in some other areas such as Eldoret. Except for Eldoret, there is no evidence to show that bulk milk has been screened for Str. agalactiae to find out the presence of mastitis due to the organism, or presence of healthy carriers acting as a source of infection with subsequent mastitis in the dairy herds. The purposes of this study were:-

(1) to investigate the occurrence of Str. agalactiae group B in the bulk milk of dairies delivering milk to the Kenya Co-operative Creameries (KCC)

(2) to find out the distribution of the herds found to be harbouring the organism

(3) to suggest diagnostic and control methods based on the results of the investigation and

(4) to attempt to improve the sensitivity of the selective medium used for the detection of CAMP positive Str. agalactiae in heavily contaminated bulk milk

REVIEW OF LITERATURE

1.   STREPTOCOCCUS AGALACTIAE

Since the discovery of chain-forming cocci in the late
nineteenth century, numerous publications have been written
about Str. agalactiae and mastitis. A brief history, classi-
fication and characteristics of Str. agalactiae and its role
in bovine mastitis is presented below. This is based on the
information gathered by Little et al. (1946a), National
Mastitis Council (1969), Merchant and Packer (1971), Schalm
et al. (1971), Deibel and Seeley (1974), Wilson and Miles (1975)
and Patterson and Hafeez (1976).

(a) Brief History and Classification

In 1873, Rivolta described chain-forming cocci in pus from
cases of strangles in the horse. Billroth and Ehrlich in
1877 applied the term streptococcus to a chain-forming coccus
they had seen in infected wounds. Ogston in 1881 showed
that micrococci were associated with infection in man.
Fehleisen in 1883 described a chain-forming coccus as the
cause of erysipelas in man. In 1887, Nocard and Mollereau
produced experimental mastitis in a cow and a goat by
inoculating into the udder a streptococcus they had iso-
lated from cases of mastitis. Kitt in 1893 proposed the
name Streptococcus agalactiae contagiosae.

Streptococcus mastitidis also appears in literature. Today,
Streptococcus agalactiae is the widely accepted species nomen-
clature for all Streptococci which can be assigned to Lancefield's
group B by serological methods. There are at least four sero-
types, namely Ia, Ib, II and III based on the reactivity of
carbohydrate antigens (S-substance) in the cell envelope.

(b) Characteristics of Str. agalactiae

They are gram-positive spherical or ovoid cells which
divide in one plane to form chains of seldom less than four
cells and frequently very long. They produce no spores and
are non-motile. They are aerobic or facultatively anaerobic.
Temperature optimum for growth is about 37°C. Colonies on
blood agar are convex and translucent and seldom > 1 mm
in diameter. Some strains produce a yellow, orange or brick
red pigment and pigment production may be enhanced by the
addition of starch or through aerobiosis. Some strains are
beta-hemolytic with a narrow zone of clear lysed cells.
A double zone of hemolysis has been described. Approximately
half of the strains are beta-hemolytic. Other strains
are either alpha-hemolytic or non-hemolytic. Except for a
few, all strains are CAMP positive, that is, capable of
enhancing hemolysis of erythrocytes which have been exposed
to staphylococcal β-toxin. In liquid media, flocculent
growth is produced at the bottom of the tube with a
relatively clear liquid above. Longer chains are

TABLE 1

DIFFERENTIAL CHARACTERISTICS OF STREPTOCOCCI MOST COMMONLY CAUSING BOVINE MASTITIS

(National Mastitis Council, 1969)

ORGANISM	LANCEFIELD GROUP	HEMOLYSIS	HYDROLYSIS OF		ACID PRODUCTION IN BROTH CONTAINING:						
			Esculin	Sodium Hippurate	Lactose	Sucrose	Salicin	Mannitol	Raffinose	Inulin	Trehalose
Primary importance											
Str. aglactiae	B	β (narrow) α γ	−	+	+	+	±	−	−	−	+
Str. dysgalactiae	C	α γ	−	−	+	+	−	−	−	−	+
Str. uberis	E/neg**	α γ	+	+	+	+	+	+	−	+	+
Secondary importance											
Str. pyogenes	A	β (moderate)	±	−	+	+	+*	−*	−	−	+
Str. zooepidemicus	C	β (wide)	±	−	+	+	+	−	−	−	−
Str. species	G	β (wide)	±	−	+	+	+	−	−	−	±
Str. species	L	β (wide)	−	±	±	+	±	−	−	−	+

* Usual reaction

** Extracts of some strains of Str. uberis react with group E antisera but do not induce group E antibodies in rabbits

formed in liquid media than on blood agar. A few strains
grow in presence of 6.5% sodium chloride. Carbohydrates
are fermented with production of acid and never with gas (table 1).
Ammonia is produced from arginine. Sodium hippurate is hydro-
lysed but not esculin and the catalase test is negative.
Most strains grow on 40% bile agar.

2. REVIEW OF METHODS USED FOR ISOLATION AND IDENTIFICATION OF STR. AGALACTIAE FROM BULK MILK SAMPLES

(a) Cultural Methods

According to the recommendations of the National Mastitis
Council (1969), the plating methods in general use for the
microbiological diagnosis of bovine mastitis are not quanta-
tive, but are based on identification of specific micro-
organisms. The following combinations have been proposed as
suitable:-

(1) 0.01 ml of milk on $\frac{1}{4}$ or 1/3 of a 10 cm plate

(2) 0.025 ml of milk on 1/3 or $\frac{1}{2}$ of a 10 cm plate

(3) 0. 1 ml of milk delivered by pipette on a whole
    10 cm plate.

Munch-Petersen and Christie (1947) used quarter milk
diluted 1:100 or more in saline in pour plating to ensure
a convenient number of isolated colonies. An inoculum size
of 0.05 ml was used by Greer and Pearson (1973) for streak-
ing on $\frac{1}{2}$ petri dish containing modified Edwards medium
(Oxoid) and 5% bovine blood. Postle (1968) used the

streak method and one loopful calibrated to deliver 0.01 ml
of milk. It has been pointed out (National Mastitis Council,
1969) that bacteria in the milk are swept up with the fat
into the cream layer and held within clumps of fat globules.
Milk should therefore be warmed up to 25°C before mixing
and taking of the inoculum. Centrifugation of milk and
plating of sediment has been reported to enhance recovery
of Str. agalactiae from milk than direct plating (Mattic
et al., 1941; Schulte, 1972; Weisner and Hubler, 1975).

After leaving the udder of the cow, milk is subjected
to a lot of contamination from the exterior of the animal
especially exterior of the udder and the adjacent parts,
manure, soil,water and air (Frazier, 1967). To exclude
some airborne contaminants which produce reactions similar
to CAMP reaction has been reported to be difficult (Christie
and Graydon 1941). Blood agar is widely used for culturing
milk samples but it is recommended for only carefully
sampled milk, otherwise contaminants overgrow the organisms
causing infection. The use of modified Edwards medium
(Oxoid) selective for streptococci and even better thallium-
crystal violet-toxin (TKT-medium 'Hauge and Ellingsen, 1953)
has proved very valuable in identifying Str. agalactiae in
bulk milk samples. The TKT-medium helps in selecting Str.
agalactiae from the primary isolation plates due to CAMP
reaction and its inability to split esculin (Hauge and
Ellingsen, 1953; Postle, 1968; Smith and Johnson, 1971).

Using TKT and CAMP positive/esculin negative criteria for
identifying Str. agalactiae, Postle (1968) observed that
streptococci which are not Str. agalactiae but which produce
a CAMP positive reaction present a problem of identity in a
single step culture system. Of these organisms, CAMP
positive Str. uberis would be expected to occur with the
greatest frequency. Further tests, biochemical or serological
should therefore be carried out to identify the organism.

Engebretsen (1971) used TKT-medium for primary isolation
and CAMP-esculin plate to register CAMP positive/esculin
negative pattern. He reported that all the 610 CAMP positive/
esculin negative streptococcal strains reacted with anti-
group B serum (Lancefield's hot-hydrochloric acid extraction
method), thus fully supporting the CAMP positive/esculin
negative criteria for identifying group B.

(b) CAMP-reaction

Following an outbreak of scarlet fever in a country district
in Australia in 1944, milk was suspected as the vehicle of
infection. While examining this milk for hemolytic
streptococci, Christie et al. (1944) discovered the ability
of Str. agalactiae to hemolyse ovine erythrocytes which had
been altered by staphylococcal β-toxin. They suggested
the use of this phenomenon as an aid in the identification
of Str. agalactiae.

Murphy, et al. (1952) called the phenomenon CAMP
(Christie - Atkins - Munch - Petersen) reaction. A number
of investigators (Munch-Petersen and Christie, 1947; Hauge

and Ellingsen, 1953; Hellman et al., 1971) tried to find out
the suitability and reliability of the CAMP reaction in detect-
ing and identifying Str. agalactiae in bulk milk and quarter
milk. It was adopted in 1947 as a routine practice in the
New York Mastitis Control Programme (Murphy et al., 1952).
Blobel (1977) pointed out that CAMP test is the best routine
method for identifying Str. agalactiae. Darling (1975) described
it as a prompt and reliable procedure for the presumptive identi-
fication of group B streptococci when a candle jar atmosphere
was used during incubation.

β-hemolytic, α-hemolytic and non-hemolytic strains of Str.
agalactiae show a CAMP reaction (Christie et al., 1944).
Engbretsen (1971) observed that 71.6% of the strains of Str.
agalactiae which were CAMP positive were non-hemolytic.

A number of factors have been reported to interfere with
the CAMP reaction. Undiluted milk often causes a confusing
darkening of the medium (Munch-Petersen and Christie, 1947).
While carrying out bacteriological examination of asceptically
drawn mastitis quarter milk samples, diluted 1:10, 1:100 or
more to ensure a convenient number of isolated colonies, Munch-
Petersen and Christie (1947) observed that addition of crystal
violet with either sodium azide or thallium acetate into the
medium and the presence of other CAMP negative microorganisms
did not prevent or interfere with the CAMP reaction. Increase
in intensity of the CAMP reaction by aerobiosis and addition
of esculin into the medium has been reported (Christie et al.,
1944 and Murphy et al., 1952). Only ovine and bovine blood
are suitable for the CAMP reaction (Christie et al., 1944).

Human, horse, rabbit or guinea pig blood are not suitable. Recently, synergistic hemolysis of human and guinea pig red blood cells by Clostridium perfringens alpha-toxin and CAMP factor from streptococci group B has been described (Steven Gubash, 1978).

Not all sheep or cattle, however, have blood suitable for the CAMP reaction because of the presence of high anti-β-toxin levels (Munch-Petersen et al., 1945; Munch-Petersen and Christie, 1947; Murphy et al., 1952). One way to avoid the effect of possible presence of anti-β-toxin is to costantly use washed erythrocytes instead of whole blood (Munch-Petersen and christie, 1947; National Mastitis Council, 1969).

Munch-Petersen et al. (1945) examined 200 strains of group B streptococci for CAMP reaction and found all of them to be positive. Later it was found that not all Str. agalactiae are CAMP positive. Murphy et al. (1947) examined 203 Lancefield serological group B streptococcal cultures and 99% were found to be positive. In 1951, the same workers examined a further 322 cultures of which 96.6% were CAMP positive. Ivashura (1972) tested 177 strains and 96.6% were positive.

The CAMP reaction does not hold absolute specificity for group B streptococci. Certain non-hemolytic staphylococci have been reported to produce hemolysis in an area

covered by staphylococcal β-toxin (Christie and Grydon, 1941). As far as the manifestation of CAMP reaction by streptococci other than streptococci group B is concerned, the conditions under which the plates are incubated have been shown to influence results. Under anaerobic conditions, group A streptococci has been reported to be CAMP positive (Esseveld et al., 1958; Darling, 1975). A few strains of Str. dysgalactiae have been reported to produce a CAMP reaction (Wilson and Slavin, 1950; Murphy et al., 1952; Hellman et al., 1971). Streptococci of Lancefield groups P, U and some group E and Str. Uberis have been found to react in CAMP tests, producing hemolytic zones comparable to those of Str. agalactiae (Thal and Obiger, 1969; Shuman et al., 1977). Thal and Obiger (1969) emphasized the importance of using other tests to avoid a false diagnosis. False positives could be avoided by incubating plates aerobically or under candle jar atmosphere (Darling, 1975). It has been stated that combination of CAMP reaction and pigment production would detect over 99% of streptococci group B (Jokipii and Liisa Jokipii, 1976). Pigment production however, requires anaerobic incubation and non-hemolytic streptococci do not produce pigment (Fallon, 1974; Jokipii and Liisa Jokipii, 1976).

The substance produced by Str. agalactiae which interacts with the erythrocytes already altered by staphylococcal β-toxin to produce a CAMP-reaction is referred to as the CAMP factor (Brown et al., 1974; Patterson and Hafeez, 1976).

Christie et al. (1944) found CAMP factor to be an
extra-cellular, filtrable substance which was inactivated
by heating at 56°C for 30 minutes, but reactivated by heating
to 100°C. Brown et al. (1974) confirmed these findings. They
found the CAMP factor to be antigenic and capable of eliciting
the production of neutralizing antibodies in rabbits and
intramammarily infected cows.

(c) Staphylococcal β-Toxin

Instead of streaking β-toxic staphylococcus on a blood
agar plate to produce β-toxin which will diffuse and render
the erythrocytes susceptible to the CAMP factor, a number of
workers have shown that sterile β-toxin can be produced
and successfully used in primary isolation of CAMP
positive streptococci by applying it on the surface of, or
incorporating into the medium (Munch-Petersen and Christie,
1947; Hauge and Ellingsen, 1953; Hansen and Winther, 1953;
Jasper and Dellinger, 1968; Greer and Pearson, 1973).
Use of paper disks impregnated with the β-toxin (CAMP-
disks) has also been described (Wilkinson, 1977).

Munch-Petersen and Christie (1944) produced β-toxin
by growing a staphylococcus S 32a (Bryce and Rountre, 1936)
which produced only β-toxin in nutrient broth containing 0.1%
agar and 0.1% glucose and incubating at 37°C for 3 days
in an atmosphere of 20% oxygen and 80% carbon dioxide.
Hauge and Ellingsen (1953) incubated the β-staphylococci for
4-6 days at 37°C. They found that carbon dioxide did not
increase β-toxin production and filtration was not necessary.

Jasper and Dellinger (1968) produced β-toxin by incubating aerobically strains of β-staphylococci in brain infusion broth, pH 7.4 for 4 days. Clarification was done by centrifuging at 1200 x G for 30 minutes. The supernate was then filtered through E.K Seitz filter and stored at 4°C.

Heat treatment has also been used for sterilizing the toxin. Hansen and Winther (1953) heated the toxin in a waterbath up to 85°C and reported complete sterilization. At 80°C, not all the staphylococci were killed. At 90°C, some toxin was destroyed. Hauge and Ellingsen (1953) heated the toxin at 82-85°C. The toxin will keep its potency even when heated to 96°C in a waterbath and then cooled down (Bakken, personal communication, 1977).

It has been shown that once prepared and sterilized β-toxin can keep for a long time at 4°C. Munch-Petersen and Christie (1947) observed that β-toxin stored for 5 years was as suitable as freshly prepared toxin. Hansen and Winther (1953) found that it remained stable for $2\frac{1}{2}$ years. Jasper and Dellinger (1968) reported it to be stable for many months at 4°C.

(d) Thallium-Crystal violet-Toxin (TKT)-medium

The need to utilise the CAMP phenomenon (Christie et al., 1944) for presumptive identification of Str. agalactiae during primary isolation led Hauge and Ellingsen (1953) to introduce thallium sulphate-crystal violet-toxin (TKT)-medium by incorporating staphylococcal β-toxin into modified Edwards medium, selective for streptococci. Thallium sulphate (1:750,000)

suppresses growth of coliforms, crystal violet (1:300) inhibits growth of staphylococci and other gram-positive cocci. Esculin (0.1%) which is also present helps in differentiating Str. agalactiae from esculin splitting streptococci. Hauge and Ellingsen (1953) observed that the selectivity of TKT-medium was especially valuable for the culture of bulk milk samples which were likely to produce profuse bacterial growth on non-selective blood agar. They further pointed out its suitability for demonstrating group B streptococci in both single and pooled milk samples.

Hansen and Winther (1953) reported an increase in the recovery of Str. agalactiae from infected milk. They also reported that by employing TKT-medium for pooled milk samples, the number of infected herds detected increased by about 50%. Postle (1968) reported a similar observation. The TKT-medium was found to be twice as effective in isolating Str. agalactiae from bulk milk samples compared to non-selective media. CAMP positive streptococci were identified in 434 herd samples with the TKT-medium as compared with 232 with a non-selective culture method.

Difficulties involved in differentiating Str. agalactiae from other streptococci on the surface of TKT-medium have been reported (Postle, 1968). CAMP positive, esculin negative streptococci on TKT-medium might be regarded as Str. agalactiae while they might be strains of Str. dysgalactiae (Wilson and Slavin, 1950; Hellman et al. 1971). Postle (1968) has also pointed out the difficulty of distinguishing CAMP positive

Str. uberis from Str. agalactiae because the darkening of
the medium around esculin splitting colony seems to depend
on intact red blood cells and CAMP positive colonies are
surrounded by a zone of clear lysis. Further tests are
therefore necessary to identify Str. agalactiae.

Addition of ferric citrate into the TKT-medium has been
reported to be useful in helping to identify Str. agalactiae
by enhancing of the CAMP-reaction and esculin splitting
(Smith and Johnston, 1972; Greer and Pearson, 1973; House and
Badakhst, 1975). Their observations contradicted the views
of Postle (1968).

Different batches of β-toxin have different toxin
content and each batch must therefore be examined to find
out the optimal concentration to use. The right type of
blood (Christie et al., 1944) must also be used in order
for the CAMP reaction to be manifested in the TKT-medium.
Hauge and Ellingsen (1953) investigated the effects of crude
toxin concentrations in the broth in the 1% to 5% range (v/v).
Hansen and Winther (1953) tried 0.5%. 1%, 2% and 4% of β-toxin
and obtained the best results with 1-2%. Complete hemolysis
occurred when 4-5% concentrations were used. Postle (1968) used
5% sheep blood and 0.02g (0.2%) of purified betatoxin per litre
with good results. It has been shown by Jasper and Dellinger
(1968) that crude β-toxin is as good to use as the purified
toxin when streaked on the surface of the medium or when
incorporated into it.

(e) Serological Grouping

Before 1933, cultural methods and biochemical tests formed the basis for the characterization and identification of different species of streptococci. Lancefield (1933) described the precipitation method for grouping streptococci. This serological method is based on the fact that different strains of each group of streptococci possess common wall antigens which react with a group specific antiserum.

The precipitation method of Lancefield (1933) which employs hot-hydrochloric acid extraction of the antigens is the classical method for grouping streptococci. A new slide co-agglutination technique using protein A-containing staphylococci sensitized with specific antibodies was described by Kronvall (1972) who used this method for typing pneumococci. Christensen et al. (1973) adopted the method of Kronvall for the grouping of streptococci. The co-agglutination method is based on a unique characteristic of protein A of certain strains of Staph. aureus. Protein A is associated with wall-components of staphylococci and has the ability to combine strongly with the Fc part of immunoglobulin G (IgG). When group-specific antiserum is added to stabilised staphylococci, the specific antibodies are adsorbed and become oriented with their antigen-combining sites directed outwards (Kronvall, 1972). Tebutt et al. (1976) coated the staphylococci using the method of Christensen et al. (1973) with a few modifications.

Streptococci are prepared for the co-agglutination reaction by growing a pure culture in a suitable medium. Todd-Hewitt broth (Oxoid) has been used successfully by several workers (Kronvall, 1972; Christensen et al., 1973; Arvilommi, 1976; Harn and Nyberg, 1976; Hryniewicz et al., 1976). Some workers have used the broth culture without trypsinization (Arvilommi, 1976) while others have trypsinized the bacterial sediment for one hour at 37°C (Christensen et al., 1973; Hryniewicz et al., 1976; Saxegaard, 1977). The sediment has also been used without trypsinization (Harn and Nyberg, 1976). Trypsinization however, provides a more homogeneous suspension of the streptococci (Beate Perch and Kjems, personal communication, cited by Christensen et al., 1973). Sometimes it eliminates cross-reactions (Tebutt et al., 1976). It has been pointed out (Christensen et al., 1973) that trypsinization of group B and D is not necessary because they do not possess surface-associated cell wall protein. Arvilommi (1976) reported that group B streptococci were more easily groupable by the co-agglutination method without prior trypsinization than by the precipitation method; 95% were groupable without trypsinization whereas 60% of other streptococci required trypsinization.

Comparative grouping by precipitation and co-agglutination methods has been done. Christensen et al. (1973) reported complete agreement with both methods. Harn and Nyberg (1976) correctly identified 98.7% of the streptococci group A, B, C and G by co-agglutination. The two methods were found not to agree in 2 out of 126 cases (Arvilommi, 1976). Saxegaard (1977) grouped 200 strains of mastitis streptococci by the two methods and obtained identical results with 95.5% of the

strains. Certain strains have been encountered which could not be grouped by the precipitation method but were groupable by the co-agglutination method and vice versa (Arvilommi, 1976; Hryniewicz et al., 1976; Saxegaard, 1977). Greer et al. (1978) compared four methods of grouping streptococci group B, that is Lancefield's precipitation test (LP), counter immunoelectrophoresis (CIE), immunofluorescence staining (FA) and slide co-agglutination test (CA). Of the 106 isolates tested, 90.56% were positive using LP test and 100% using CIE, FA and CA tests. CIE, FA and CA tests showed false positives, but LP test did not.

The commercial group antisera used for coating staphylococci are not always specific. An antiserum may contain antibodies for more than one group (Lindqvist, personal communication, 1978).

Some disadvantages associated with the co-agglutination method have been reported. The most disturbing one is cross-reactions between groups. Christensen et al., (1973) did not detect any cross-reactions between various groups, A, B, C, D and G strains. Using undiluted anti-streptococcal sera to sensitize the Cowan I strain of Staph. aureus, Hryniewicz et al. (1976) reported cross-reactions between group B and G. Diluting group A antisera I: 16 completely abolished cross-reactions. Weak cross-reactions usually occurring after 1 minute and which were not specific were reported between groups (Ham and Nyberg, 1976). Tebbutt et al. (1976) reported cross-reaction between some strains of group A against group B antiserum by co-agglutination. Saxegaard (1977) observed cross-

reactions between groups A and C, B and G and B and L. It has been reported that cross-reactions can be eliminated by absorption or dilution of the antiserum (Hryniewicz et al., 1976, Tebbutt et al., 1976). The so called cross-reactions could be due to the presence of more than one group of streptococci in the prepared antigen (Greer et al., 1978).

Cross-reactions probably occur due to the immunological relationship between the respective C-carbohydrates (Krause, 1963, Curtis and Krause, 1964). The polysaccharide antigen of group A is composed of N-acetylglucosamine and rhamnose. Terminal N-acetylglucosamine is the antigenic determinant (Schmidt, 1952; Krause, 1963; McCarty, 1958; cited by Wilson and Miles, 1975). Rhamnose is the determinant sugar in groups B and G polysaccharides and the two groups exhibit cross-reactions (Kurtis and Krause, 1964). The antigenic determinant for group L is N-acetylglucosamine attached to rhamnose oligosaccharide. This accounts for the partial cross-reaction between groups A and L (Karakawa et al., 1971).

The co-agglutination method has the advantage of being cheap, rapid, reliable and easy to perform (Christensen et al., 1973; Tebbutt et al., 1976; Harn and Nyberg, 1976; Saxegaard, 1977; Arvilommi et al., 1978). Recently, Arvilommi et al., 1978) have been able to group correctly 70 out of 71 Strepto-coccal strains by growing a pure colony, picked directly from a primary plate, in 0.5 ml of Todd-Hewitt broth for $2\frac{1}{2}$ hours instead of 24 hours and trypsinizing for 30 minutes. Richard Rosner (1977) successfully grouped 92.6% (119/132) of β-hemolytic streptococci after 4 hours incubation in Todd-Hewitt

broth using Phadebact Streptococcus Test (Pharmacia Diagnostics, Piscataway, N.J.). Only 0.1 ml of specific group antiserum is required to coat 1 ml of a 10% suspension of protein A-containing staphylococci which is made to 1% final concentration and only one drop of this is required to mix with one drop of streptococci for a test. In positive cases, co-agglutination occurs within 30 seconds with corresponding reagents. Reaction occurring within one minute is usually taken as positive.

3. CELL COUNT, CALIFORNIA MASTITIS TEST (CMT) AND THE RELATIONSHIP TO STR. AGALACTIAE INFECTION

Cell count in milk refers to cells which comprise leucocytes derived from the blood and the epithelial cells from the mammary ducts and acini (Zlotnick, 1947; cited by Wright, 1977). Schalm et al. (1971) have enumerated the various types of cells included in the cell count. A threshold of 500,000 cells per ml of bulk milk has been proposed to distinguish normal from subclinical mastitic milk (Tolle, 1975; Wright, 1977).

Electronic cell count (ECC) tests are considered to be the best methods available at present (Pearson, 1971). The California Mastitis Test (CMT) (Schalm and Noorlander, 1957) is routinely used as a means of estimating the number of cells in the milk. Good correlation between CMT and ECC has been reported (Schalm and Noorlander, 1957; Pearson, 1971). CMT is based on the capability of anionic detergents to break

open the nuclei of cells releasing deoxyribonucleic acid (DNA) which causes a sharp rise in viscosity. The degree of viscosity depends on the number of cells present in the milk. Sodium laurylsulfate in a concentration of 3-5% is one of the detergents which has been found useful. Other detergents used in the soap industry are as effective. Bromcresol purple in 1:10,000 concentration is used as the pH indicator. Usually, mastitis milk has an elevated pH and the pH indicator (bromcresol purple) shows a deep purple colour which accompanies the viscosity (Jaartsveld,1963; Schalm et al., 1971).

Madsen et al. (1976) reported finding positive correlation between frequency of mastitis and cell counts of bulk milk. Several other workers have reported positive correlation between elevated cell counts and the isolation of Str. agalactiae. Postle (1968) used a modification of Prescott and Breed (1910) to count leucocytes. A relationship was demonstrated between leucocyte numbers in excess of 500,000 per ml and the presence of CAMP-positive streptococci in both bulk milk and quarter milk samples.

The average cell count of quarters infected with Str. agalactiae has been reported to be higher than those infected with Staph. aureus (Postle et al., 1971; Wright, 1977). A good correlation between Str. agalactiae isolations and the cell count was reported by Greer and Pearson (1973). A correlation coefficient of 0.75 was obtained. In further support of this, Pearson et al. (1976) reported a very high correlation (0.849 correlation coefficient) when they examined

526 herds with 37.6% being positive for Str. agalactiae. Many
factors which influence the number of cells in milk have
been reported, but inflammation of the udder has the greatest
influence (Wright, 1977). In a review of literature,
Wright (1977) gave the factors influencing the number of
cells in milk as (1) inflammation of the udder (2) high
ambient temperature (3) stage of lactation (4) treatment
with corticosteroids (5) mechanical injury (6) dietary
change e.g. turning out to grass (7) regression of the
udder as a result of disease (8) stress and (9) the age of
the animal. He concluded that the relationship between the
bulk milk cell count on one hand and mastitis prevalence and
bacterial isolations on the other hand, can never be a good
one. This was in agreement with the observations of Renner
(1975) and Giesecke (1975). Giesecke (1975) observed that
increased cell count in milk was not pathognomonic for clinical
mastitis.

4. RESERVOIR, ENVIRONMENTAL CONTAMINATION AND SOURCES OF
   INTRA-MAMMARY INFECTION

   The infected bovine udder is considered to be the only
natural reservoir of Str. agalactiae (Little et al., 1946a;
Philpot, 1975; MacDonald, 1977) and in human beings, the
female genital tract is the primary reservoir (Patterson and
Hafeez, 1976). However, Str. agalactiae has been isolated
from many other sites: teat lesions, air, bedding, milking
equipment, milkers' hands and other objects. Their presence

in such sites is a consequence of contamination with infected milk. In the absence of intramammary infection, they disappear from the secondary sites in about 3 weeks (Harrison, 1941; Philpot, 1975).

Hughes (1953) and Havelka (1974) observed that for the spread of mastitis in a herd, milk is the most dangerous source of Str. agalactiae. The organism however, dies very quickly on healthy or eroded skin (Neave et al., 1969; Jackson 1970; MacDonald, 1977).

Group B streptococci which do not grow at $37^{\circ}C$ and give negative CAMP reactions were reported causing infection among captive tropical fish (Robinson and Meyer, 1966; cited by Wilson and Miles, 1975).

5. STR. AGALACTIAE AND BOVINE MAMMARY INFECTION

In 1887, Nocard and Molleraue were the first to produce mastitis in a cow and a goat by inoculating the udder with Str. agalactiae isolated from cases of mastitis (Stableforth, 1959; Schalm, et al., 1971). Of the three streptococci important in bovine mastitis (Str. agalactiae, Str. dysgalactiae and Str. uberis), Str. agalactiae is the most prevalent (Little et al., 1946a; Stableforth, 1959; Schalm, et al., 1971). It has been pointed out that 85% of the chronic conditions observed in dairy cattle are due to streptococci and 80% of these are caused by Str. agalactiae (Little et al., 1946a).

Available literature indicates that passage of the organisms
through the streak canal is the only way of intramammary
infection. Attempts to infect cows by drenching with
infected secretions or injecting Str. agalactiae subcutaneously
or intravenously have been unsuccessful. Milkers' hands,
udder cloths and milking machine liners infect the udders
via the teat canal (Stableforth, 1959; Neave et al., 1969;
Neave and Jackson, 1971). Calves have been suggested as
occasional transmitters if they suckled both infected and clean
cows or one another (Klein and Klechner, 1941; Little et al.,
1946b; cited by Stableforth, 1959). Flies have also been
incriminated. Bryan et al. (1940) reported recovery of strepto-
cocci of mastitis origin from the udder tissue of heifers
which had never lactated. In support of the preceeding reports,
Schalm (1942) showed that Str. agalactiae can actually colonize
the developing mammary gland of heifers after cross suckling,
with recovery of the organisms at first lactation. Infection
often follows introduction of a small number of the organism
into the teat beyond the streak canal (Newbould et al., 1965).
It has been reported that the incidence of mammary infection
due to Str. agalactiae in an uncontrolled herd increases with
age (Schalm et al., 1971).

Viral infections, staphylococcal infections, anatomy and
physiology of the dairy cow have been mentioned as predis-
posing the cow to infection with Str. agalactiae (Weitz, 1971).
Colonization of the udder tissue produces chronic mastitis
which is subject to periodic exacerbations and the mammary
gland undergoes fibrosis and atrophy (Weitz, 1971; Schalm,

1977). Cultural latency and intermittent shedding of Str. agalactiae are complicating factors in the diagnosis, and eradication of the infection (Little, et al., 1946a).

Resistance of the udder to colonization with pathogens following an infection with non-pathogens was reported by Forbes (1970). Induced leukocytosis was suggested to be responsible for the resistance. Findings of Linde et al., (1975) and Bramley (1978) gave an added support to these observations. When udder quarters were infected with Staph. epidermidis and then challenged with Str. agalactiae (Bramley, 1978), it was found that 88.9% of the nine Staph. epidermidis infected quarters did not get infected but 90.9% of the eleven uninfected quarters became infected. Again, resistance was suggested to be due to an elevated number of polymorphonuclear cells. Reiter and Oram (1967) pointed out that high numbers of polymorphonuclear cells protect the udder against infection with staphylococci or streptococci irrespective of the presence of complement or antibodies.

6. STR. AGALACTIAE AND HUMAN DISEASE

Over the last few decades group B streptococci have gained increasing recognition as the etiological agent of human diseases, particularly septacemia and meningitis in the newborn. The newborn is presumed to acquire the disease from the infected maternal genital tract. It has been reported that human and bovine strains of Str. agalactiae are distinct

(Ghoroury, 1950; Butter and deMoor, 1967; Herbert Braunstein
et al., 1969; Baker et al., 1973; Darling, 1975; Franciosi
et al., 1973; Prakash et al., 1973; Baker and Barret, 1974;
Patterson and Hafeez, 1976).

Certain serological and biochemical types of group
B streptococci have been reported to be predominating in
animals, but there is a broad area of overlap between the
strains from these two hosts (Tolle, 1975; MacDonald, 1975).
Simmons and Keogh (1940) have shown that human strains are
more virulent for mice and unable to ferment lactose,
whereas the less virulent bovine strains usually are
fermenters. Norcross et al. (1976) found no entirely
consistent differences in their attempts to define distin-
guishing characteristics between isolates of human and
bovine origin. Evidence to show that bovine strains of Str.
agalactiae are involved in human disease is lacking
(MacDonald, 1977). Butter and deMoor (1967) were able to
isolate human and not bovine strains of Str. agalactiae from
throats of dairy workers in the Netherlands.

In the Federal Republic of Germany it was found that
the incidence of group B streptococci isolated from hospital
patients correlated closely to the consumption of raw milk
(Vorzugsmilch). Twenty per cent of "Vorzugsmilch" in the
Fed. Rep. of Germany was found to contain group B streptococci
(Tolle, 1975).

Dodd et al. (1977) are of the opinion that in the
modern industry where milk is heat treated, a justification
for control measures for public health reasons does not arise.

MATERIALS AND METHODS

1. BULK MILK

(a) Description of the areas and places of sampling

The areas with a high density of dairy cows producing
a lot of milk for marketing were considered appropriate
for this study. These are concentrated in the highlands
at an altitude between 1,500-2,700 meters and receiving
an average annual rainfall of 625 mm (Maina Wanjigi, 1972).
The following areas were selected:-

(1) Nairobi area comprising of the dairies within Nairobi
itself, dairies in Kiambu district, dairies in Machakos
district and other contiguous areas.

(2) Mt. Kenya region comprising of the dairies supplying
milk to the Kenya Co-operative Creamery (KCC), Kiganjo.

(3) The Rift Valley Province and the adjacent Nyandarua
district, Central Province, comprising of dairies supplying
milk to Naivasha, Nakuru, Nyahururu, Eldoret and Kitale KCCs.

The herd sizes vary considerably from a few thousand
heads to five or even fewer cows. Delivery of milk to the
creameries is done either individually or through
Co-operative societies. This study was aimed at covering
both small and big herds. Kenya Co-operative Creameries
were therefore found to be the most appropriate centers
for sample collection.

Milk starts arriving at the creameries by 7.00 in the morning every day. It is delivered in aluminium cans by lorries or vans under no refrigeration and received at the delivery platform. Sampling was started with the first deliveries early in the morning.

(A) Sampling of the milk

Unless otherwise stated, all the equipment used in this study, was washed and sterilized using hot air oven at 160°C for one hour.

Sampling materials included (1) sterilization set comprising of a portable gas cooker and two water boiling cans (2) sterile universal bottles (3) sterile sampling spoons and (4) Coleman cool-box with frozen ice-packs.

Milk was sampled from the cans on delivery, as they travelled along the conveyor belt. The milk in the can was thoroughly stirred with a spoon sterilized in boiling water and approximately 2 ml withdrawn. The sampling spoon was rinsed in hot water and then placed in boiling water while another spoon was being used for sampling the next can. Milk of one supplier was pooled to make a sample of 10-20 ml. For a supplier with more than 10 cans, 10 cans were pooled to make one sample. From their records, the managers of the creameries supplied information on the identity of the milk suppliers, location of the farm(s) and their estimated sizes. All the samples were kept and transported to the laboratory in a Coleman cool-box containing ice-packs. They were examined

immediately on arrival in the laboratory or after storage
at 4°C overnight.

2. CALIFORNIA MASTITIS TEST (CMT)

Milk was usually examined immediately on arrival at the
laboratory. Testing and scoring was carried out as described
by Schalm et al. (1971) except that instead of negative,
trace, weak, distinct positive and strong positive, numerical
figures 1, 2, 3, 4 and 5 were used respectively. Sometimes
the test was carried out at the sampling centers. When
it was not possible to do CMT the same day of collection,
milk was preserved at 4°C until the next day.

3. TESTING FOR BACTERIAL INHIBITORY SUBSTANCE

The punch hole technique (Johnson et al., 1977)
was used to test all the milk samples.

Mueller-Hinton agar (Oxoid) plates were flooded with
3 ml of a 24 hour culture of Micrococcus luteus (M. luteus)
in dextrose broth. Excess was drained off by using pasteur
pipettes and the plates left to dry for a few minutes. Holes
of 7 mm diameter were punched in the medium using a sterile
cork-borer. They were labelled with sample numbers. After
filling the holes with milk (sour milk was buffered first
using phosphate buffer) using sterile pasteur pipettes,
plates were left to stand for 15-20 minutes at room temp-
erature for prediffusion to take place. Incubation was at
37°C overnight. Results were read by examining the plates for

zones of inhibition and measuring their diameters. Positive
samples were heated to 82°C for 5 minutes, cooled and retested
as above to find out whether the inhibition was due to
antibiotics. This was done in pairs. One portion of the
sample was tested against penicillinase impregnated discs to
find out whether the antibiotic present was penicillin.
Results were read as follows:-

    (i) Zone of inhibition measuring 8.0 mm and above,
        after heating the milk was taken as positive for
        antibiotics.

    (ii) Concentration above 0.01 IU/ml of penicillin
        was taken as positive.

Measurements were done using a standard curve prepared
as follows: Dilutions of Benzyl penicillin sodium salt were
made and diameters of zones of inhibition measured. A
standard curve was drawn by plotting these diameters against
the logarithms of penicillin concentrations. Concentration
of 0.01 IU/ml was found to be the lowest which could be detected
with reproducible results. To read a test, the diameter of
inhibition was measured, logarithm read from the curve and
antilogarithm (IU of penicillin) obtained from the logarithm
tables.

4. CULTURAL METHODS

Modified Edwards medium (Oxoid) was used for culturing
the milk samples at first, but it was later replaced with
TKT-medium. The change was introduced because no

Str. agalactiae was isolated from 352 milk samples collected
from KCC Nairobi and Kiganjo by streaking them on the modified
Edwards medium. It was felt that TKT-medium was a better
alternative. On this medium, CAMP reaction due to CAMP
positive Str. agalactiae can be read directly on the primary
isolation plate, thereby facilitating the picking of Str.
agalactiae colonies.

(a) Preparation of the TKT-medium

This was prepared using modified Edwards medium, crude
β-toxin and washed bovine calf blood as follows: To 250 ml
of melted modified Edwards medium cooled down to 50-45°C,
20 ml of the washed blood were added followed by 5 ml (1.8% v/v)
of the crude β-toxin. It was used immediately after mixing.
Ingredients of the TKT-medium were prepared as described below.

Edwards medium (modified)

This was prepared according to the instructions of the
manufacturer (Oxoid) one or two days prior to the sampling of
milk and stored in 250 ml amounts at 4°C.

Collection and washing of blood

Bovine calf blood was aseptically collected and
defibrinated by shaking with glass beads. It was then tested
for sterility by incubating 1 ml mixed with blood agar base
at 37°C overnight. The sterile blood was aseptically distributed
into test-tubes and centrifuged at 2,000 r.p.m. for 10 minutes.

Serum was discarded and an equal volume of sterile saline
(0.85% NaCl) added to the erythrocytes and the test tube
inverted several times for the first washing. The erythrocytes
were washed three times and sterile saline was added to the
packed cells to reconstitute the original volume, preserved
at 4°C and used within 2-4 days.

Preparation of crude staphylococcal β-toxin

An Australian strain of β-toxic Staph. aureus producing
a wide zone of altered bovine red blood cells was obtained
from National Mastitis Laboratory, Oslo.

One isolated pure colony was inoculated into 250 ml of
Brain Infusion Broth (Oxoid) and incubated at 37°C for 5 days.
The broth was shaken every day at least once, and clarified
by filtration and centrifugation. The filtrate containing
the toxin was collected in 50 ml amounts. It was sterilized
by heating in a waterbath until the temperature of the toxin
reached 92°C mark. After cooling, it was tested for sterility
by inoculating two drops into 10 ml of Todd-Hewitt broth
(Oxoid). Incubation was done at 37°C for 1 to 2 days. If any
of the flasks showed turbidity, indicative of growth of bacteria,
they were discarded. Sterile toxin was stored in the
refrigerator at 4°C.

Titration of the β-toxin

β-toxin was titrated to find out the optimal concentration
to be used in the TKT-medium. Blood agar base (Oxoid) was

prepared and then washed calf blood (7-8% v/v) was added followed by a calculated volume of the crude β-toxin to produce the desired toxin concentration. Thin layers (approximately 10 ml) were poured into petri dishes. CAMP positive non-hemolytic Str. agalactiae was streaked on the surface and incubated at 37°C overnight. For each batch of toxin preparation, the following toxin percentages were tested:- 0.5, 1, 2, 3, and 4%. For the batches of crude β-toxin used in this study, 1 and 2 percent toxin were found to give very good CAMP reaction. It was therefore decided to use 1.8% in the TKT-medium.

(b) Inoculation of milk samples

Surface streaking

In the beginning, 352 Nairobi and Kiganjo samples were examined by streaking them on the surface of the modified Edwards medium containing 5% unwashed bovine blood. The samples were first incubated overnight at 37°C prior to streaking. After streaking, all the plates were incubated as described above. Two suspect colonies (based on the colonial characteristics) were picked and tested for the CAMP reaction on a blood agar plate. This was followed by grouping using Lancefield's Precipitation method.

Pour plating

Pour plating method using TKT-medium was adapted to
examine the 906 samples from the Rift Valley Province and
the adjacent district of Nyandarua, Central Province,
following the failure to recover Str. agalactiae from the
previous 352 samples using the streak method.

Refrigerated samples were warmed up to room temperature
and then thoroughly mixed by shaking. About 0.1 to 0.2 ml of
milk were mixed with 0.5 ml of sterile saline (0.85% NaCl) in
a petri dish. Approximately 10 ml of the TKT-medium were
poured into the petri dish so as to form a thin layer. The
inoculum was then mixed with the medium by gentle shaking
and rocking in a horizontal plane. The plates were incubated
in inverted positions at 37°C overnight. They were then
examined for colonies producing wide zones of clear hemolysis,
presumably due to the CAMP reaction.

CAMP/Esculin tests

The presumptive CAMP positive colonies were tested further
for CAMP reaction and esculin splitting ability by replating
them on blood-esculin-agar plates. The plates contained
7-8% (v/v) washed calf blood and 0.1% esculin. A straight
wire loop was used to pick the colonies at the centre of
the hemolytic zones. β-toxic Staph. aureus was streaked
across the centre of each plate and a few implants made

where the streptococci had been streaked to enable manifesta-
tion of the CAMP reaction. CAMP reactions were read after
overnight incubation of the plates at 37°C.

5. SEROLOGICAL GROUPING

All the isolates found to be CAMP positive and esculin
negative were presumptively identified as Str. agalactiae
and later subjected to serological grouping. CAMP positive/
esculin positive isolates were taken to be Str. uberis.

(a) Lancefield's Precipitation Method

A pure colony of streptococci to be grouped was inocula-
ted into 10 ml of dextrose broth (Oxoid) and incubated over-
night at 37°C. Bacterial sediment was obtained by centrifuging
the broth at 2,000 r.p.m. for 10 minutes and decanting the
supernatant. The cell wall antigens were extracted using the
hot-hydrochloric acid method of Lancefield (1933) as described
by Facklam (1974).

The capillary precipitation test was carried out using
the antigen extract, Wellcome streptococcal group antisera
and hematocrit capillary tubes.

The outside of a capillary tube was wiped clean with
tissue paper and one end dipped into the antigen extract and
let to fill by capillarity up to 1/3. The capillary tube was
then wiped clean on the outside to prevent contaminating the
antiserum with the antigen. The same end of the capillary
tube was placed into the antiserum of the group being

tested for and removed when a volume equal to that of the antigen had entered the capillary tube. The capillary tube was then planted in the plasticine with the column of antigen layered over the column of antiserum.

The reaction was read after 5 to 10 minutes and after 15 to 30 minutes (those tubes which did not show reaction after 10 minutes).

(b) Grouping by the Slide Co-agglutination Method

Coating of the protein A-containing Staph. aureus with streptococcal group specific antiserum

A 10% suspension, in phosphate-buffered-saline (PBS) pH 7.3, of formaldehyde and heat-treated protein A-containing Staph. aureus strain Cowan I (NCTC 8530) prepared according to the method of Kronvall (1972) was obtained from the National Institute of Public Health, through the National Mastitis Laboratory, Oslo. Streptococcal group specific antisera were obtained from Wellcome Research Laboratories, Beckenham, England.

One millilitre of the 10% suspension of the Staph. aureus was delivered into a tipped centrifuge tube and centrifuged for 15 minutes at 3,000 r.p.m. The supernatant was decanted and the sediment resuspended in 0.9 ml of PBS and 0.1 ml of the appropriate group antiserum added. Vigorous mixing was done immediately using a shaker. The tubes were then left to stand at room temperature for 5 to 10 minutes after which they were centrifuged for 15 minutes at 3,000 r.p.m.

The supernatant (containing residual antibodies) was collected
and kept at 4°C. The sediment was then washed 3 times
with PBS. The washed sediment was eventually resuspended
in 5 ml of PBS containing 0.1% of sodium azide to make a 2%
final suspension. Using the supernatant from the above
coating, it was possible to coat a second batch of
staphylococci. Tubes were let to stand a little bit
longer (10-15 minutes).

Preparation of streptococci for grouping

One pure colony of the streptococci to be grouped was
inoculated into 10 ml of Todd-Hewitt broth (Oxoid) and
incubated at 37°C overnight. The broth was centrifuged
at 3,000 r.p.m. for 15 minutes. Supernatant was decanted
into test tubes and preserved. The sediment was resuspended
in 0.5 ml of 0.2M tribuffer, pH 8.0 containing 5 mg %
trypsin (beef pancreas extract, BDH Biochemicals Ltd.
Poole, England). The mixture was then incubated at 37°C
for one hour.

The slide test

One drop of the trypsinized sediment of the strepto-
cocci was placed on a microscope slide. A drop of the 2%
suspension of protein A-containing Staph. aureus Cowan I
coated with the streptococcal group specific antiserum, was
placed next to it. The two were then mixed with an applicator

stick and the slide rocked to and fro continuously
for up to one minute. The mixture was examined for
co-agglutination within this time. Culture super-
natants were also examined in the same way.

6. FACTORS AFFECTING THE CAMP REACTION

TKT-medium is a selective medium for streptococci and an indicator of CAMP positive streptococci, mainly Str. agalactiae. When milk is cultured in this medium, CAMP positive Str. agalactiae can be distinguished from the other streptococci because of its CAMP reactivity. This medium has been very successfully used for isolating Str. agalactiae from bulk milk in many countries of the world. It was because of this fact that the TKT-medium was selected for examining bulk milk samples for Str. agalactiae in this investigation.

In the course of these investigations it was noticed that a large proportion of the TKT-medium plates had a heavy growth of contaminants and were severely discoloured (browning) after overnight incubation at 37°C. The contaminants were identified as streptococci, the majority of which were capable of splitting esculin. Growth of β-hemolytic non-streptococcal organisms was also noticed. It was difficult to read CAMP reactions under these conditions. The overall number of samples positive for Str. agalactiae, out of 906, was very low (1.3%). In Kenya bovine mastitis has been reported to be a common disease of some importance, but the prevalence of Str. agalactiae of mastitis has not been documented throughout the country (Kariuki, 1978, personal communication; Lauerman et al., 1973; Ward, 1977, personal communication). In Eldoret where some investigation had been done, the prevalence of Str. agalactiae was shown to be fairly high (Kariuki, 1978). Also in this investigation, the highest percentage of samples positive for Str. agalactiae came from Eldoret.

In view of the above findings, further tests which are described below, were carried out to reassess the method before making any conclusions. Some other experiments aimed at improving the CAMP reaction and TKT-medium are also described.

Effect of Bacterial Contamination of Raw Bulk
Milk on the CAMP Reaction

Raw milk was collected from KCC Nairobi. A non-hemolytic strain of Str. agalactiae obtained from the Mastitis Laboratory, Oslo and which gave a good CAMP reaction was grown in Todd-Hewitt broth (Oxoid) and used as the test organism.

Amounts of 0.2 ml of raw milk diluted to 1/2, 1/4, 1/8 and 1/16 in sterile saline (0.85% Nacl) and 0.2 ml of undiluted milk were cultured in the TKT-medium containing Str. agalactiae. The Str. agalactiae culture was diluted to 10^{-7} so as to give few isolated colonies per plate. A TKT-medium control containing Str. agalactiae and no milk was also set up. All the plates were incubated at 37°C overnight after which they were examined for the CAMP reaction.

Isolation of fecal streptococci

A homogenate of a severely discoloured TKT-medium in which bulk milk had been cultured, was prepared in sterile saline. This was streaked on Slanetz medium (Oxoid) and incubated at 37° and 44°C separately. Heavy growth of streptococci with characteristics of fecal streptococci was noted at both temperatures. The isolate split esculin and was found to belong

to streptococci group D.

Diluted Todd-Hewitt broth culture of the fecal streptococci isolate and the CAMP positive Str. agalactiae were mixed and cultured in the following media to find out what effect this had on the CAMP reaction: (a) blood agar (BA) containing 2% β-toxin and 7-8% washed bovine red blood cells (BRBCs) (b) BA containing 2% β-toxin, 7-8% washed BRBCs and 0.1% esculin and (c) BA containing 2% β-toxin, 7-8% BRBCs, 0.1% esculin and 1% maltose. A control using fecal streptococci from our reference stock was also set up. All the plates were incubated at $37^{\circ}C$ overnight after which they were examined for CAMP reactions and changes in the medium. Diameters of the CAMP reaction zones were measured to the nearest tenth of a millimeter using a calibrated magnifying glass.

Bacterial Count in Bulk Milk

Total count in the bulk milk samples was done to estimate the load of contamination which would interfere with the reading of the CAMP reaction.

Five raw milk samples from KCC Nakuru, were diluted in sterile saline up to 10^{-7}. One tenth of a millilitre (0.1 ml) of the dilutions 10^{-3} to 10^{-7} was cultured in Plate Count Agar (PCA) containing 0.1% esculin. Esculin was incorporated so as to help to identify esculin splitters. All the plates were incubated at $37^{\circ}C$ overnight. This temperature was preferred because the main interest was to count those bacteria growing at the temperature used for culturing

milk samples when examining for Str. agalactiae. Esculin
splitting colonies (colonies surrounded by a brown zone) and
non-esculin splitting ones were counted. To be able to detect
the brown colour around esculin splitting colonies, a black
background was found to be most suitable.

Effect of Heated Milk, Maltose and Lactose on the CAMP Reaction

Raw bulk milk was collected from KCC Nairobi and divided
into two portions. One portion was heated to $80^{O}C$ for 2
minutes to kill vegetative bacteria The other portion was
left untreated. A series of separate plates were prepared
in which 0.2 ml of raw milk, 0.2 ml of heated milk, 1%
maltose and 1% lactose were mixed with the TKT-medium and
used for culturing the CAMP positive non-hemolytic Str.
agalactiae as shown in the plan below.

1. TKT-medium + 1% maltose

2. "   "   +   "   + raw milk

3. "   "   +   "   + heated milk

4. "   "   + heated milk

5. "   "   + 1% lactose

6. TKT-medium alone (control).

All the plates were incubated at $37^{O}C$ overnight and examined
for the CAMP reactions. Diameters of the CAMP reaction zones
were measured as described above.

The Effect of Incorporating Heated Milk and Maltose
in the TKT-medium for the Detection of CAMP Positive
Str. agalactiae in Bulk Milk

Brown et al. (1974) reported enhancement of CAMP reaction
by maltose. This was confirmed in our experiments. Heated milk
was also found to enhance the reaction. An attempt was therefore
made to incorporate these two ingredients into the TKT-medium
and then use it to examine bulk milk samples for CAMP positive
Str. agalactiae.

Twelve bulk milk samples were collected from KCC Nakuru.
Amounts of 0.2 ml of each sample diluted to 1/2 and undiluted,
were cultured by pour plating in (a) TKT-medium alone (b)
TKT-medium plus 1% maltose and (c) TKT-medium plus heated milk.
Non-hemolytic CAMP positive Str. agalactiae alone was cultured
in the above 3 media as control.

Effect of Varying Concentrations of Maltose
on Sizes of CAMP Reaction Zones

Weighed amounts of maltose were dissolved in appropriate
volumes of melted TKT-medium to make final concentrations of
0.1%, 0.5%, 1%, 2%, 3%, 4%, 5% and 6% (w/v). Ten millilitres
of medium containing these concentrations of maltose were mixed
in a petri dish with CAMP positive non-hemolytic Str. agalactiae
and then cultured by pour plating in the TKT-medium.

Effect of Heated Milk Whey on the CAMP Reaction

Heated milk was found to impart a cloudy (milky) background appearance to the medium. In an effort to overcome this, an experiment was carried out to investigate whether clear whey contains substance(s) enhancing the CAMP reaction.

Whey was produced by coagulating milk using acid or rennin enzyme (Chr. Hansen's Laboratorium A/S Sankt Annae Plads 3 DK-1250 Copenhagen, K Denmark). One batch of whey was prepared by coagulating 240 ml of bucket milk using N/1 hydrochloric acid. The coagulated milk was filtered, centrifuged and the whey separated. The whey was neutralized using N/1 sodium hydroxide. The neutralized whey was heated to 80°C for 2 minutes to kill vegetative bacteria. It was centrifuged again before use. Another batch of whey was prepared by coagulating 200 ml of milk using rennin enzyme. A few mg of the enzyme powder were added to the milk and dissolved by shaking. Whey was filtered and centrifuged. It was heated to 80°C for 2 minutes to kill vegetative bacteria and then centrifuged again. It was found that storage at 4°C made the whey clearer as the remaining casein settled to the bottom of the container.

Effects of various concentrations of acid-produced whey (ranging from 1 to 14%), on the CAMP reaction was investigated using TKT-medium containing 1.8% β-toxin and 7-8% washed bovine red blood cells. TKT-medium was prepared and appropriate amounts of whey and medium pipetted into petri dishes to give the final percentage of whey (v/v) in the 10 ml final volume per plate. CAMP positive non-hemolytic Str. agalactiae was

incorporated in all the plates. TKT-medium controls were set
up. After mixing, all the plates were incubated at 37°C
overnight.

Effect of Combining Heated Milk Whey with Maltose
on the CAMP Reaction

Fourteen percent of acid-produced whey which had previously
exhibited a marked increase in diameters of CAMP reaction
zones was combined with varying concentrations of maltose and
mixed with CAMP positive non-hemolytic Str. agalactiae. These
were cultured by pour plating in TKT-medium pipetted as
described earlier. Maltose and TKT-medium controls were
set up.

Using the rennin-produced whey, a checker board type
experiment was set up to find out which concentrations of
whey and maltose, when combined, give the largest CAMP
reaction zones. A control was set up for each concen-
tration of whey and maltose. TKT-medium without maltose
or whey was also set up as control. Whey and TKT-medium
were pipetted into the petri dishes as described earlier.

RESULTS

The results presented here are those of the 906
samples taken from 5 Kenya Co-operative Creameries in the
Rift Valley Province and the adjacent district of
Nyandarua, Central Province namely: Naivasha, Nakuru,
Nyahururu, Eldoret and Kitale (map appendix I). Results of
other tests carried out later are also presented. The milk
samples were examined using the TKT-medium and the slide
co-agglutination method of grouping the isolated bacteria.
Table 2 shows the number of milk suppliers and the number
of samples collected from each of the 5 creameries.

California Mastitis Test (CMT)

CMT reactions were scored 1, 2, 3, 4 and 5. Scores 1-2
were deemed negative and 3-5 positive. Clotted and sour
samples were not assigned CMT scores. It was found that
226 (24.9%) samples were CMT positive ($\geq$ 3), 370 (40.8%)
CMT negative ($\leq$ 2) and 310 (34.2%) clotted or sour.

Table 3 shows the results of the CMT. Nakuru had
the lowest percentage (0.8%) of CMT positive samples and
the highest percentage (89.7%) of clotted or sour samples.
Eldoret had a very low percentage (15.2%) of positive
samples followed by Nyahururu (34%). The highest percentage
of positive samples (47.3%) was shown by Naivasha. Table 4
shows the number of samples falling under each of the CMT
scores.

Str. agalactiae was recovered from one CMT positive

sample and 8 CMT negative samples. The highest number of
samples positive for Str. agalactiae (7/12) came from
Eldoret. Table 5 depicts the samples positive for Str.
agalactiae for each CMT score.

Bacterial Inhibitory Substances

Out of the 906 raw milk samples examined, 65 (7.2%) were
positive for bacterial inhibitory substances (sour milk
included after buffering with phosphate buffer). After
heating, 24 (2.6%) were positive for nonnatural inhibitors.
Of these, 7 (29.2%) were penicillin positive and 17 (70.8%)
other nonnatural inhibitors. Table 6 shows the number of
positive raw milk samples and the number of positive samples
after heating the milk.

Naivasha had 14.7% samples positive for bacterial
inhibitory substances before heating and 8.7% after heating.
The one sample which yielded Str. agalactiae was negative for
inhibitory substances. About 6.7% of the Nakuru samples were
positive before heating and 3.3% positive after heating. Out of
the 4 samples which were positive for Str. agalactiae group B,
3 of them were positive for inhibitory substnaces excluding
penicillin (table 7). Two were positive before heating
and one after heating. Nyahururu had 6.6% samples positive
for inhibitory substances before heating and 1.6% after
heating. Kitale and Eldoret had the lowest percentage of
positive samples for inhibitory substances after heating:
6.3% and 1.5% respectively. From Eldoret, no sample positive
for bacterial inhibitory substances yielded Str. agalactiae.

Bacterial Isolations

In the TKT-medium, bacterial colonies from 403 (44.5%) of the total 906 milk samples showed β-hemolytic zones similar to CAMP reaction. When they were plated on blood esculin agar plates, 218 (24.1%) were shown to be strepto-cocci. Details are given in table 8.

Table 9 shows the streptococcal isolations made from the 906 milk samples. Twelve (1.3%) samples yielded Str. agalactiae, 17 (0.9%) Str. uberis and 189 (20.9%) other streptococci. The number of milk suppliers and milk samples positive for Str. agalactiae are shown in table 10. No isolations were made from milk samples collected from Nyahururu or Kitale. Eldoret, Nakuru and Naivasha had 5.3%, 1.9% and 0.7% of their samples positive for Str. agalactiae respectively.

Serological Confirmation of the Presumptively Identified Str. agalactiae

Table 11 shows the results of the serological confirmation of the 12 presumptively identified Str. agalactiae isolates (CAMP positive/esculin negative) using the slide co-agglutination method. All the 12 isolates were confirmed to be group B. Trypsinized sediments of two isolates, both from Eldoret, auto-agglutinated. Their supernatants, however, agglutinated well with the staphylococci coated with streptococcal group B antiserum. The trypsinized sediment and supernatant

of the <u>Str</u>. <u>agalactiae</u> isolated from Naivasha and the trypsinized sediment of one isolate from Nakuru cross-reacted with group G antiserum. The cross-reaction was observed to be weaker than with anti-group B staphylococcal reagent for both trypsinized sediment and supernatant. The supernatant of all the 12 isolates reacted with anti-group B staphylococcal reagent. The reaction, however, was always weaker than with trypsinized sediment, but the reactions were easily readable.

Table 12 shows the suppliers whose milk was positive for <u>Str</u>. <u>agalactiae</u> group B as was confirmed by serology. The last column in the table indicates the number of samples from which <u>Str</u>. <u>agalactiae</u> was isolated and confirmed.

Only one sample (table 12) yielded a positive isolate from each supplier and hence 12 suppliers contributed the positive samples. These represent 3.8% of the total milk suppliers (317) dealt with. The 12 suppliers contributed 30 samples, 3.3% of the total.

FACTORS AFFECTING THE CAMP REACTION

Effect of Bacterial Contamination of
Raw bulk milk on the CAMP Reaction

The TKT-medium in which the raw undiluted milk
was inoculated became severely discoloured with heavy
growth of microorganisms. No hemolytic zones were
seen.

Dilution of the milk to $\frac{1}{2}$ reduced the darkening
of the medium. A few small and indistinct hemolytic
zones could be seen in a brown background. Further
dilution of milk to $\frac{1}{4}$ decreased darkening even more
and improved CAMP reaction in terms of the sizes of
zones and clarity of hemolysis. Further dilution of
milk beyond $\frac{1}{4}$ improved the brightness of the medium
and thereby also improving the clarity of CAMP
reaction.

Table 13 shows the number of CAMP reaction zones
and their diameters. No CAMP zones were visible with
undiluted milk. At $\frac{1}{2}$ dilution, zones were too small
and indistinct to be measured. The sizes of diameters
for the dilutions $\frac{1}{4}$, 1/8 and 1/16 did not differ a lot.

The heavy contamination of bulk milk and severe
discolouration of the TKT-medium were found to be due
to esculin splitting streptococci, mainly streptococci
of group D.

Test for inhibitory substances in the milk was
negative.

Effect on CAMP Reaction of Esculin-splitting
Fecal Streptococci Isolated from Bulk Milk

Heavy growth of streptococci was noted in all the plates. The blood agar containing esculin was severely discoloured. The CAMP reaction was poorly manifested or absent altogether, making it difficult to differentiate Str. agalactiae from the other strepto- cocci. Addition of 1% maltose to the blood agar containing esculin did not improve the situation. On the other hand, blood agar without esculin showed CAMP reaction, but it was noticed that even without esculin, the medium showed some discolouration.

Bacterial Count in the Bulk Milk

Two of the five samples yielded more than 300 contaminating colonies per plate (>5.74×10^9 bacteria per ml of milk). The average counts for the remaining 3 samples are shown in table 14. The average total count for the 3 samples was 5.74×10^9 bacteria per ml of bulk milk. Of these, 72.1% were esculin splitters and 27.4% non-esculin splitters. These figures show clearly that bulk milk is heavily contaminated by the time it reaches the creameries.

Effect of Heated Milk, Maltose and Lactose
on the CAMP Reaction

Incorporation of heated milk into the TKT-medium
unexpectedly improved the CAMP reaction substantially.
The effect was mainly an increase in sizes of the CAMP
reaction zones which were more than trippled in diameter
when compared with the control (Table 13). The milk,
however, imparted a cloudy (milky) background appearance
to the medium.

One percent maltose was also found to produce large
CAMP reaction zones with clear penetrating hemolysis. The
diameters of the zones averaged 5 mm with a range of 3.7-6.0
mm as compared with the control plates without maltose. The
colonies were generally larger than in the standard medium.

Combination of heated milk and maltose produced CAMP
reaction zones whose sizes were considerably larger than those
produced by heated milk or maltose alone. The hemolysis
however, was not completely clear because of the cloudy
(milky) background. Some unlysed erythrocytes were notice-
able around the bacterial colonies. The sizes of CAMP
reaction zones averaged 6.2 mm in diameter with a range of
4.0-8.4 mm as compared with 1.7 mm in the control plates
without milk.

CAMP reaction could not be read when maltose or
heated milk were combined with untreated raw milk because
of severe discolouration of the medium due to the large
number of contaminating microorganisms.

One percent lactose also increased the CAMP reaction
zones, but not to the extent produced by the addition of
heated milk, maltose or maltose plus heated milk (Table 13).

Effect of Incorporating Heated Milk and maltose in the TKT-medium for the Detection of CAMP Positive Str. agalactiae in Bulk Milk

Severe discolouration was observed in all three media
(TKT-medium alone, TKT-medium plus maltose and TKT-medium plus
heated milk) in which the undiluted raw milk was cultured.

Half dilution of the milk similarly showed severe disco-
louration of the medium, except with 5 samples out of 12.
Some faint hemolytic zones were recognizable in a background
of severe darkening in all the three media where these 5
samples had been cultured. Colonies were picked from these
zones and tested for CAMP reaction and esculin splitting. .
Three samples yielded CAMP positive, esculin negative streptococci.
Serological grouping confirmed that they were streptococci
group B. The other two samples yielded CAMP positive, esculin
positive hemolytic streptococci. They did not react with
group B antiserum.

Effect of Varying Concentrations of Maltose on Sizes of CAMP Reaction Zones

The sizes of CAMP reaction zones increased with
increasing maltose concentration up to about 1%. Beyond

1%, sizes decreased with increasing maltose concentration (table 15, figure 1 and 2). Lower concentrations of maltose ($\leq$ 1%) produced the largest CAMP reaction zones.

Effect of Heated Milk Whey on the CAMP Reaction

Heated milk whey was found to contain substance(s) enhancing the CAMP reaction. This was clearly demonstrated by the increase in diameters of CAMP reaction zones as the concentration of whey increased (Table 16, 18 and figure 1 and 2). Higher concentrations of whey produced larger CAMP reaction zones. Whey produced by acid or rennin coagulation of milk had similar effect on the CAMP reaction.

The hemolytic zones were clear. The cloudy appearance observed with the heated milk was absent.

Rennin was completely inactivated by heating to 80°C for 2 minutes.

Effect of Combining Heated Milk Whey with Maltose on CAMP Reaction

Combination of maltose and whey produced by acid and rennin coagulation of milk increased sizes of CAMP reaction zones more than the maltose alone or whey alone (Table 17, 18 and figure 1 and 2). This agreed with the earlier observations using heated milk (Table 13).

Table 18 shows diameters of the CAMP reaction zones
when whey produced by rennin coagulation of milk, and
maltose were combined at various concentrations. Earlier
on, it was found that low concentrations of maltose ($\leq$ 1%)
and high concentrations of whey ($\geq$ 10%) (figure 1), gave
the largest CAMP reaction zones. Subsequently, combination
of low concentrations of maltose with high concentrations
of whey gave maximum sizes of CAMP reaction zones (figure 1
and 2).

It can be seen (table 18) that the average CAMP
zone diameters are smaller in this experiment (control
included) as compared with the previous ones. However, if
the increase in sizes of the zones is considered with
reference to the controls for each experiment, the ratios
of increase are similar.

DISCUSSION

CALIFORNIA MASTITIS TEST (CMT)

According to Schalm and Noorlander (1957), CMT is valid
for bucket milk if conducted within 24 to 36 hours of collection.
Samples should therefore be stored under refrigeration at $4^{O}C$
to control bacterial growth. In this study, it was noticed that
although the milk was transported and preserved at $4^{O}C$, a large
number of samples were sour, particularly those from Nakuru. This
meant that CMT could not be carried out on such samples to
estimate somatic cells because the test would not be valid.

High correlation between positive CMT and Str. agalactiae
isolations has been reported (Greer and Pearson, 1973; Pearson
et al., 1976). In this study, 8 (66.7%) out of 12 samples
positive for Str. agalactiae were CMT negative (table 5). Of
these 8 samples, 7 were from Eldoret, which incidentally had a
very low number of CMT positive samples (table 3). These
findings do not agree with those of Greer and Pearson (1973)
or Pearson et al. (1976). The findings however, do
agree with the views of Bakken (1977) that elevated cell counts
in bulk milk do not correlate well with Str. agalactiae
infections in the herds. Elevated somatic cell count does not
necessarily reflect presence of pathogenic microorganisms in
the udder. Many factors other than the infectious agents
influence the number of polymorphonuclear cells in milk (Wright,
1977). Introduction or Staphylococcus epidermidis into the udder
of a cow has been shown to prevent colonization of the udder
with Str. agalactiae (Bramley, 1978). Induced leucocytosis has
been suggested to be responsible for the resistance of the udder

CONTAMINATION OF MILK

A high load of contaminating bacteria was usually encountered
in the milk samples. This might have played a role towards the
sourness of milk. A total count of $> 5.74 \times 10^9$ bacteria per
ml of bulk milk was recorded for 5 samples collected at KCC
Nakuru. Of these, 72.1% were esculin splitters and 27.4% non-
esculin splitters.

Frazier (1967) pointed out that besides the bacteria
from the interior of the udder, milk acquires a considerable
number of contaminating organisms from the exterior of the
animal during milking. In the Kenyan farms, a wide variation
exists in milking hygiene practices and handling of milk after
it has left the cow. There are farmers who use machine milking,
but the majority of farmers practise hand milking. Less conta-
mination occurs with machine milking than with hand milking
(Frazier, 1967). From the dairies milk is transported to the
creameries in cans by lorries or vans under no refrigeration.
On the spot observation at the creameries revealed that many
cans were dusty and wet with leaking milk. Preliminary inquiry
showed that evening milk was added to the morning milk by some
farmers before delivery to the creamery and in general there
are no cooling facilities available at the farms prior to
transport to creameries.

The foregoing indicates that from the time milk leaves
the cow, it acquires a heavy load of bacterial contamination as
it goes through the transport chain. Vehicles from far off
travel long distances under the heat of the sun. Quite often
vehicles stand outside the creamery waiting in the sun. These
conditions favour contamination and growth of bacteria,

especially lactose fermenters, causing lowering of the pH of milk. This would explain why many samples were clotted or sour, particularly after overnight stay although under refrigeration at 4°C. The metabolic products of these contaminants and biochemical changes spoil and render milk inferior in quality. The possible detrimental effects they may have on the health of the consumer should not be ignored, because such heavily conta- minated and spoilt milk cannot yield wholesome milk or milk products even after processing and destruction of microorganisms.

BACTERIAL ISOLATIONS

Following the failure to isolate Str. agalactiae from 352 Nairobi and Kiganjo samples using Edwards medium (modified) alone, the method was changed so that the CAMP reaction could be used as the primary criterion for the isolation of presumptive Str. agalactiae. The use of CAMP reaction in this study as the primary identifying characteristic of Str. agalactiae was based on the fact that over 96% of Str. agalactiae strains have been shown to be CAMP positive.

It cannot be said with certainty that the initial 352 samples did not contain Str. agalactiae because the random picking of two suspected Str. agalactiae colonies from the Edwards medium, based on colonial characteristics, was by no means reliable. Furthermore, attempts to group the suspect colonies by Lancefield's precipitation method produced doubtful precipitin reactions.

Some 12 samples out of 906, representing 1.3% of the
total, were diagnosed positive for Str. agalactiae using TKT-
medium, CAMP reaction and co-agglutination method of grouping.
Trypsinized sediments of two group B streptococcal isolates
auto-agglutinated. Their supernatants however, reacted well
with group B antiserum. This coupled with CAMP and esculin
tests confirmed that the streptococci were Str. agalactiae
group B. Earlier on, Christensen et al. (1973) had observed
auto-agglutination with group B.

Cross-reactions were also observed. Trypsinized sediment
and the corresponding supernatant of one isolate cross-reacted
with group G antiserum. Rhamnose has been shown to be the
determinant sugar in both group B and G polysaccharides and that
the two exhibit cross-reactions (Kurtis and Krause, 1964).

The percentage of samples positive for Str. agalactiae
(1.3%) was very low. A wide variation of occurrence was noted
in the areas studied. Prior to this study, a high prevalence
of Str. agalactiae had been noted in Eldoret (Kariuki, 1978).
This was as a result of the examination of bulk milk samples
(from KCC Eldoret) followed by individual quarter samples of
the herds whose bulk milk yielded the organism. In this study,
it was also found that the highest number of positive samples
(7/12) came from Eldoret (table 9). Many points can be raised
in connection with this low percentage of samples. The simplest
conclusion is to assume that these figures represent the real
prevalence of Str. agalactiae in the bulk milk. But this has to
be weighed heavily against several factors.

The milk samples might have had inhibitory factors to the CAMP factor, for example antibodies (Brown et al., 1974) which might have neutralized the activity of the CAMP factor before it could act on the β-toxin-modified erythrocytes. Milk samples also might have contained antibodies to the β-toxin from Staph. aureus. These antibodies might have been able to interact with the β-toxin-modified erythrocytes in such a way that CAMP factor was unable to exert its full effect on the erythrocytes. It cannot be ruled out that the antibacterial substances did not have an effect on Str. agalactiae isolation from those milk samples where they were present. It was found that 64 milk samples (7.2%) out of 906 were positive for antibacterial substances and out of these, 7 (9%) contained penicillin. No Str. aglactiae was isolated from samples containing penicillin, but was isolated from 3 Nakuru samples positive for other antibacterial substances (table 6). Resistance of streptococcal mastitis to penicillin, streptomycin and tetracycline has been reported (Kariuki, 1977). However, Str. agalactiae is very sensitive to penicillin and resistance to this antibiotic has never been shown (Schalm, 1977).

The high dilution of infected milk containing Str. agalactiae when mixed with normal milk would reduce chances of its recovery. Postle (1968) noted that for Str. agalactiae to be detected in a herd on one occasion, at least 5% of the quarters must be infected for the organism to be isolated from the bulk milk.

FACTORS AFFECTING THE CAMP REACTION

When the bulk milk samples were cultured in the TKT-medium, changes in the medium which could have intefered with the CAMP reaction and hence recovery of Str. agalactiae were observed. Many samples showed severe discolouration of the TKT-medium, heavy growth of contaminating microorganisms mainly esculin splitting streptococci and β-hemolytic gram negative short rods. The darkening of the medium was mainly due to the split esculin. Experiments done using raw undiluted milk, raw diluted milk and fecal streptococci isolated from the bulk milk showed that reading of the CAMP reaction under such conditions was difficult or even impossible and probably many samples positive for Str. agalactiae might have escaped detection.

In the experiments, the CAMP reaction was practically impossible to read with undiluted raw milk but was easily readable when milk was diluted to 1/4 and more. The dilution of milk apparently diluted out the esculin splitters. Brightness of the TKT-medium was substantially improved. Although dilution of milk could be contemplated in order to reduce discolouration of the medium, this would reduce the sensitivity of the test because Str. agalactiae would also be diluted out decreasing its chances of detection. At the moment, it is also difficult to think of a substance which could inhibit growth of fecal streptococci, which were found to be mainly responsible for the discolouration of the TKT-medium, without inhibiting Str. agalactiae. Presence of other hemolytic organisms in a severely discoloured TKT-medium adds to the difficulties of reading the CAMP reaction.

The gram negative contaminants were those which were resistant to thallium sulphate and crystal violet at the concentrations used in the TKT-medium. No further study was done to characterise these organisms. Although they did not inhibit the CAMP reaction, their β-hemolysis could easily be confused and interpreted as a CAMP reaction. An increase in CAMP reaction beyond the β-hemolysis of these contaminants, would therefore be of great advantage. CAMP positive Str. agalactiae would be easily distinguished from β-hemolytic organisms. It was therefore decided to investigate factors which would facilitate the distinction between β-hemolytic zones and CAMP reactions.

Experiments which were carried out using various concentrations of maltose showed enhancement of the CAMP reaction by maltose (table 15, 18 and figure 1 and 2). This was in agreement with the earlier observations by Brown et al. (1974). Brown et al. (1974) attributed the enhancement of the CAMP reaction to the large amounts of CAMP factor produced due to the utilization of maltose by the growing Str. agalactiae. This shows that the sensitivity of TKT-medium can be improved by incorporating maltose. In the course of these investigations, low concentrations of maltose (≤1%) produced the largest CAMP reaction zones. The sizes of zones decreased with increasing maltose concentration beyond 1%. This indicates that use of more than 1% maltose in the medium would not be of any advantage.

Milk heated to 80°C for 2 minutes and incorporated in the medium also enhanced the CAMP reaction. Whey was found to contain substance(s) responsible for the enhancement of the CAMP reaction. What causes enhancement of the CAMP reaction in this case is not readily explainable. Probably it is a combination of several factors. Experiments with lactose did not show large increase in sizes of CAMP reaction zones compared to those produced by maltose or whey (table 13, 15, 16, 18). Whey produced no cloudy background appearance in the TKT-medium. It is therefore better than the heated whole milk.

Whey produced by acid and rennin coagulation of milk produced similar effects on the CAMP reaction (table 16, 18 and figure 1 and 2). Coagulation of milk with rennin was most convenient.

The increase in sizes of the CAMP reaction zones by the combination of maltose and heated milk (table 13) or whey (table 17 and figure 1 and 2), more than by any of the three alone suggests a synergistic activity. Again, to explain how this synergism works needs further investigation.

Results obtained with the checker board type experiment show smaller diameters of CAMP reaction zones (table 18) when compared with the previous experiments using maltose, whey and a combination of both. However, it is also noticeable that the average diameter of the CAMP reaction zones in the TST-medium control is in this case also smaller (1.1 mm) than those of the earlier experiments (1.7 mm and 2.0 mm). The discrepancy is

eliminated if the ratio of increase in diameters is considered with reference to the TKT-medium controls in each case.

From an economic point of view, whey would be cheaper to use in the medium than maltose. Of advantage is the fact that a smaller amount of commercial medium base would be used per plate when whey is included. The volume of whey used in the course of these investigations did not dilute the medium to such an extent that agar could not solidify and hold to the plates. However, of concern is the fact that inhibitory substances, thallium and crystal violet, may be diluted to such an extent that they no longer exert their full effect in inhibiting bacteria other than streptococci. The problem could be overcome by increasing their concentrations to compensate for the dilution factor. Combination of 1% maltose with 2-5% whey (figure 2) gives fairly large CAMP reaction zones, bigger than with maltose alone or whey alone, although less than when 1% maltose is combined with 10% or more of whey. The latter combination is therefore recommended for the TKT-medium.

Although the incorporation of maltose and/or whey into the TKT-medium would be of great advantage, it was observed that in our samples of heavily contaminated milk, the addition of maltose, heated milk or whey does not improve the readability of the CAMP reaction. However, the increase in sizes of the CAMP reaction zones by the addition of maltose, whey or a combination of both, would be of distinct advantage in areas where the use of the TKT-medium is not impaired by heavy

contamination of the bulk milk samples. Under conditions
of low contamination, the larger CAMP reaction zones obtained
by the medium developed in the course of this investigation,
will facilitate the detection of the CAMP positive organisms,
since the β-hemolytic zones of CAMP negative organisms do not
show any increase in size in this medium.

CONCLUSIONS

Greer and Pearson (1973) and Pearson et al. (1976)
reported a good correlation between positive California
Mastitis Test (CMT) and isolations of Str. agalactiae from
milk. No correlation was established with our samples. The
test was not useful in detecting samples positive for Str.
agalactiae .

A high total bacterial count per ml of milk and a high
number of sour and clotted milk samples were observed. These
showed that bulk milk is heavily contaminated by the time it
arrives at the creameries. The metabolic products of the
contaminants and biochemical changes spoil and render milk
inferior in quality prior to processing in the creameries.
Such poor quality milk cannot be expected to yield wholesome
milk or milk products after processing and should therefore
be viewed as a potential public health hazard.

Edwards medium (modified) is not the best medium for
isolating Str. agalactiae because there is no specific
characteristic to distinguish it from the other streptococci.
TKT-medium is better because CAMP reaction can be used to
identify the CAMP positive Str. agalactiae in the primary
isolation medium. In this study, no Str. agalactiae was
recovered using modified Edwards medium, but was recovered
using TKT-medium and the method of pour plating. The organism
was recovered from milk sampled at Naivasha, Nakuru and
Eldoret creameries, all in the Rift Valley Province. Eldoret
had the highest number of positive samples followed by Nakuru

and Naivasha.

Since infected bovine udder is considered to be the only natural reservoir of Str. agalactiae (Little et al.,1946a; Philpot, 1975; McDonald, 1977), recovery of Str agalactiae from the bulk milk indicated presence of mastitis in the herds or healthy carriers. The number of positive samples (1.3%) was however, very low. Further examination of the reliability of the method indicated that this figure may be much higher.

Esculin splitting fecal streptococci were found to be the major contaminating bacteria. These caused severe dis-colouration of the TKT-medium, which made the reading of CAMP reactions difficult and often impossible. Therefore, an unknown number of Str. agalactiae positive samples might have passed undetected. It was found that TKT-medium was not suitable for screening heavily contaminated milk for CAMP positive Str. agalactiae. The prevalence of Str. agalactiae mentioned here must therefore be considered a gross under-estimation.

Further tests on the medium showed that CAMP reaction is expressed well when the contamination by esculin splitting streptococci and the discolouration of the TKT-medium is low. Maltose and heated milk greatly enhance CAMP reaction, but heated milk imparts a cloudy (milky) background to the medium. The whey from heated milk contains substance(s) which enhance(s) CAMP reaction. Heated whey can replace heated milk thus eliminating the cloudy background in the medium. Combination of maltose with heated milk or whey has a synergistic effect

·on the increase in sizes of the CAMP reaction zones and
addition of maltose or heated milk or whey to the TKT-medium
or blood agar containing esculin does not improve the reading
of CAMP reactions when the milk sample is heavily contaminated.

These investigations thus resulted in the development
of a medium which specifically enhances the hemolytic zones
of the CAMP reactions, while the hemolytic zones produced by
CAMP negative organisms remain unaltered. Such a medium will
retain the selective properties of the original TKT-medium for
streptococci and will show a clear distinction between true
CAMP positive organisms and other hemolytic organisms.

The following are suggestions based on the findings in
this study.

1. Bulk milk is heavily contaminated by the time it arrives
   at the creamery. Extension work to educate the farmers on
   dairy management practices, milk hygiene, milk handling
   and storage should therefore be emphasized so as to minimise
   the degree of contamination of milk. This is important
   because poor quality milk will not give a good end product
   which is presented to the consumer.

2. Sampling of milk from moving cans at the creameries is
   difficult because the cans run fairly fast along the
   conveyor belt. Probably sampling at the dairies is a better
   alternative.

3. Since severe discolouration of the TKT-medium has been
   shown to interfere with the reading of the CAMP reaction,
   and that this discolouration is mainly due to the splitting

of esculin, addition of esculin in the TKT-medium is not
recommended.

4. The inclusion of maltose or heated whey or both would
increase the sensitivity of the TKT-medium when specifically
looking for the CAMP positive Str. agalactiae.

Table 2: Number of milk suppliers and milk samples
collected from each creamery.

KCC	NUMBER OF SUPPLIERS	NUMBER OF SAMPLES
NAIVASHA	49	150
NAKURU	82	252
NYAHURURU	97	182
KITALE	38	190
ELDORET	51	132
TOTAL	317	906

Table 3: CMT results of 906 milk samples collected from 5 Kenya Co-operative Cremearies

KCC	TOTAL	CMT POSITIVE (SCORE ≥ 3)		CMT NEGATIVE (SCORE ≤ 2)		CLOTTED OR SOUR	
		No.	%[*]	No.	%[*]	No.	%[*]
NAIVASHA	150	71	47.3	64	42.7	15	10
NAKURU	252	2	0.8	24	9.5	226	89.7
ELDORET	132	20	15.2	112	84.8	0	0
NYAHURURU	182	62	34	55	30.2	65	35.7
KITALE	190	71	37.4	115	60.5	4	2.1
	906	226	2?.9	370	40.8	310	34.2

[*] Percentages in the columns have been rounded off. They may therefore not add up to 100.

Table 4: Distribution of samples among the CMT scores for each creamery.

KCC	CMT SCORE RATING									CLOTTED OR SOUR		TOTAL
	NEGATIVE					POSITIVE						
	1		2		3		4		5			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
NAIVASHA	13	8.7	51	34	60	40	11	7.3	0	0	15	10	150
NAKURU	8	3.2	16	6.3	2	0.8	0	0	0	0	226	89.7	252
NYAHURURU	13	7.1	42	23	49	27	12	6.6	1	0.5	65	35.7	182
ELDORET	74	56	38	28.8	19	14.4	1	0.8	0	0	0	0	132
KITALE	79	41.6	36	18.9	56	29.5	15	7.9	0	0	4	2.1	190
	187		183		186		39		1		310		906

Table 5: Number of samples positive for Str. àgalactiae
compared with the CMT scores.

KCC	CMT					CLOTTED OR SOUR	STR. AGALACTIAE TOTAL
	NEGATIVE		POSITIVE				
	1	2	3	4	5		
NAIVASHA	0	0	0	1	0	0	1
NAKURU	0	1	0	0	0	3	4
NYAHURURU	0	0	0	0	0	0	0
KITALE	0	0	0	0	0	0	0
ELDORET	3	4	0	0	0	0	7
TOTAL	3	5	0	1	0	3	12

Table 6: Fractions of samples showing inhibition before
and after heating.

	INHIBITION			
	RAW MILK SAMPLES	AFTER HEATING RAW SAMPLES SHOWING INHIBITION $(82^{o}C, 5$ MIN.$)$	IDENTI- FIED AS PENICI- LLIN	UNIDENTI- FIED
NAIVASHA	22/150 (14.7%)	13/22 (59.1%)	3	10
NAKURU	17/252 (6.7%)	5/17 (29.4%)	3	2
NIAHURURU	12/182 (6.6%)	3/12 (25%)	1	2
KITALE	12/190 (6.3%)	1/12 (16.7%)	0	1
ELDORET	2/132 (7.2%)	2/2 (100%)	0	2
TOTAL	65/906 (7.2%)	24/65 (22.9%)	7	17

Table 7 : Sources of milk samples which were positive for bacterial inhibitory
substances and Str. agalactiae.

KCC	SOURCE OF SAMPLE	SAMPLE No.	RAW MILK	HEATED MILK	PENICILLIN	OTHERS	STR. AGALACTIAE
NAKURU	NAKURU SF (166, 508)*	18	+	+	–	+	+
"	NJORO LF (76)*	133	+	–	–	–	÷
"	RONGAI- NJORO LF (607)*	178	+	–	–	–	+

SF = Small farm(s)   LF = Large farm(s)   * KCC suppliers number

Table 8 :Number of samples examined, samples which showed ß-hemolytic zones on the TKT-medium and results of the CAMP and esculin tests of those organisms.

KCC	SAMPLES EXAMINED	NUMBER OF SAMPLES SHOWING HEMOLYTIC ZONES ON THE TKT-MEDIUM	CAMP AND ESCULIN TESTS (ON THE BLOOD ESCULIN AGAR PLATE)				β-HEMOLYTIC NON-STREPTOCOCCI SHOWING NEGATIVE CAMP AND ESCULIN REACTIONS
			STREPTOCOCCI				
			CAMP + / ESCULIN -	CAMP + / ESCULIN +	CAMP - / ESCULIN -	CAMP - / ESCULIN +	
NAIVASHA	150	92	1	2	32	25	32
NAKURU	252	78	4	1	3	27	43
NYAHURUR	182	94	0	0	7	24	62
KITALE	190	54	0	0	0	24	30
ELDORET	132	85	7	14	3	44	17
TOTAL	906	403	12	17	45	144	184

Table 9: Streptococcal isolations made from the milk samples.

KCC	NUMBER OF SUPPLIERS	NUMBER OF SAMPLES EXAMINED	POSITIVE ISOLATIONS		
			STR. AGALACTIAE	STR. UBERIS	OTHER STREPTOCOCCI
NAIVASHA	49	150	1	2	57
NAKURU	82	252	4	1	30
NYAHURURU	97	182	0	0	31
KITALE	38	190	0	0	24
ELDORET	51	132	7	14	47
	317	906	12	17	189

Table 10 : Percentage of milk suppliers and milk samples
positive for Str. agalactiae

KCC	NUMBER OF SUPPLIERS	SUPPLIERS WITH MILK POSITIVE FOR STR. AGALACTIAE		SAMPLES EXAMINED		
				TOTAL NUMBER OF SAMPLES	POSITIVE	
	No.	No.	%	No.	No.	%
NAIVASHA	49	1	2	150	1	0.7
NAKURU	82	4	4.9	252	4	1.9
NYAHURURU	97	0	0	182	0	0
KITALE	38	0	0	190	0	0
ELDORET	51	7	13.7	132	7	5.3
TOTAL	317	12	3.9	906	12	1.3

Table 11: Serological grouping of the presumptive _Str._
        agalactiae isolates (CAMP positive/escilin negative)
        using the slide co-agglutination method.

KCC	ISOLATE SAMPLE NUMBER	CO-AGGLUTINATION REACTION WITH ANTI-GROUP B REAGENT	
		TRYPSINIZED SEDIMENT	SUPERNATANT
NAIVASHA	117	+*	+*
NAKURU	18	+	+
"	133	+*	+
"	178	+	+
"	189	+	+
ELDORET	14	+	+
"	32	+	+
"	54	+	+
"	92	+	+
"	108	auto-agglutination	+
"	120	+	+
"	124	auto-agglutination	+

* Cross-reaction with group G.

Table 12 : Suppliers of milk which yielded Str. agalactiae
group B.

KCC	MILK SUPPLIER	SAMPLES EXAMINED	STR. AGALACTIAE POSITIVE SAMPLES
ELDORET	Mayo farm	1	1
"	Sociani small scale farmers	5	1
"	Sergoit small scale farmers	3	1
"	Yamumbi farm	4	1
"	Ngecheck farmers (Nandi) (108)*	5	1
"	Kipkabus (553, 1968)*	1	1
"	Kabongo farm (12)*	1	1
NAKURU	Nakuru small scale farmers (166, 508)*	1	1

Table 12 continued.

KCC	MILK SUPPLIER	SAMPLES EXAMINED	STR. AGALACTIAE POSITIVE SAMPLES
NAKURU	Rongai-Njoro (80)*	1	1
"	Ndodori FCS (Matindiri) (429)*	1	1
NAIVASHA	Njambini FCS (452)*	6	1

* KCC supplier's number

FCS = Farmers' Co-operative Society

Table 13 : Diameters of CAMP reaction zones in the TKT-medium
containing raw (contaminated) bulk milk, diluted
raw bulk milk, 1% maltose, heated milk, Heated
milk plus 1% maltose and 1% lactose.

COMPOSITION OF MEDIUM	NUMBER OF ZONES MEASURED	ZONE DIAMETERS (MM)	
		RANGE	AVERAGE
TKT-medium alone (control)	40	1.3-2.0	1.7
TKT-medium with:			
1. Raw bulk milk:-			
Undiluted	No CAMP reactions		
1/2 diluted	Very indistinct unmeasurable hemolytic zones		
1/4 "	40	2.0-3.5	2.6
1/8 "	40	1.9-3.4	2.6
1/16 "	40	1.5-3.0	2.2
2. 1% maltose	40	3.7-6.0	5.0
3. Heated milk	40	4.4-7.0	5.6
4. Heated milk + 1% maltose	37	4.0-8.5	6.2
5. 1% lactose	40	2.5-4.5	3.1

Table 14: Total bacterial count in bulk milk samples
after overnight incubation at 37°C.

SAMPLE	ESCULIN SPLITTERS $\times 10^9$	NON-ESCULIN SPLITTERS $\times 10^9$	TOTAL COUNT $\times 10^9$
1	4.23	1.275	5.505
2	1.81	1.29	3.1
3	6.35	2.26	8.61
Total	12.39	4.725	17.215
Average number / ml of milk	4.13	1.575	5.738
% count out of the total (5.74×10^9)	72.1%*	27.4%*	
4 More than 5.74×10^9 per ml of milk			
5 " " " " " " "			

* Figures are rounded off and do not therefore add
up to exactly 100.

Table 15: Diameters of the CAMP reaction zones at
various concentrations of maltose after
overnight incubation at 37°C.

CONCENTRATION OF MALTOSE % (w/v)	NUMBER OF ZONES MEASURED	ZONE DIAMETERS (MM)	
		RANGE	AVERAGE
TKT-medium control	56	1.9-2.2	2.0
0.1	42	2.8-4.0	3.5
0.5	38	3.2-4.6	3.9
1	45	3.2-4.5	3.8
2	48	2.5-4.2	3.3
3	45	3.0-4.1	3.6
4	41	3.2-4.0	3.7
5	44	2.8-3.5	3.1
6	46	2.4-3.5	3.1

Table 16 : Diameters of the CAMP reaction zones at various
concentrations of whey (acid-produced and
neutralized) after overnight incubation at 37°C.

CONCENTRATION OF WHEY % (v/v)	NUMBER OF ZONES MEASURED	ZONE DIAMETERS (MM)	
		RANGE	AVERAGE
TKT-medium control	56	1.9-2.2	2.0
1.9	48	2.8-3.5	3.2
3.8	43	3.0-3.8	3.4
5.7	41	3.0-4.0	3.6
7.6	37	3.3-4.0	3.8
9.4	41	3.0-4.0	3.8
11.3	39	3.6-4.5	4.0
14.1	37	3.7-5.0	4.2

Table 17: Diameters of CAMP reaction zones when 14% of
whey (acid-produced and neutralized) was
combined with varying concentrations of maltose
and incubated overnight at 37^OC.

CONCENTRATIONS OF MALTOSE AND WHEY		NUMBER OF ZONES MEASURED	ZONE DIAMETERS (MM)	
			RANGE	AVERAGE
MALTOSE % (w/v)	WHEY % (v/v)			
TKT-medium control	14	9	1.8-2.3	2.0
0.1	14	9	3.0-4.0	3.8
0.5	14	7	4.5-4.8	4.6
1	14	9	4.5-5.0	4.8
2	14	11	4.0-5.0	4.6
3	14	13	4.0-5.0	4.3
4	14	12	4.0-5.0	4.2
5	14	9	4.0-4.5	4.1
6	14	8	3.5-4.0	3.7

Table 18: Average diameters and ranges of the CAMP reaction
zones when whey (rennin-produced) and maltose were
combined at various concentrations.

CONC. OF WHEY % (v/v)	CONCENTRATION OF MALTOSE % (w/v)						
	0	0.5	1	2	3	4	5
0	1.1 1.0-1.5	2.2 2.0-2.5	2.8 2.5-3.0		2.3 2.0-2.5	2.2 1.9-2.6	2.3 2.0-2.6
1	2.0 1.7-2.4	3.1 2.4-3.6	2.7 2.0-3.0		2.2 2.0-2.7	2.3 1.8-2.8	2.2 2.0-2.5
2	2.2 2.0-2.5	2.8 2.2-3.6	3.3 2.8-3.8		2.0 2.0-2.3	2.5 2.0-3.0	2.2 2.0-2.6
3	2.4 2.0-3.0	2.7 2.2-3.2	3.4 2.8-4.0		1.8 1.5-2.0	2.1 1.8-2.5	2.0 1.6-2.5
4	2.6 2.4-3.0	2.4 2.0-2.6	2.6 2.3-3.0		1.9 1.8-2.0	2.2 2.0-2.5	2.2 1.8-2.6
5	2.6 2.3-3.0	3.3 2.5-3.8	3.3 2.6-3.8		2.3 2.0-2.9	2.3 2.0-2.8	2.2 2.0-2.8
6	2.7 2.5-3.5	2.9 2.7-3.5	3.0 2.5-3.0		2.8 2.5-3.6	2.4 2.0-2.8	2.4 1.8-2.9
7	2.8 2.2-3.0	3.3 3.0-4.0	3.4 3.0-4.0		2.8 2.0-3.0	2.7 2.0-3.0	2.6 2.0-3.0
8	3.0 2.6-3.0	3.2 3.0-3.5	3.7 3.0-4.0		3.0 2.6-3.5	2.7 2.4-3.0	2.7 2.0-3.0
9	2.7 2.4-3.0	3.6 3.0-4.0	4.0 3.0-4.3		2.6 2.2-3.0	2.4 2.0-2.8	2.3 2.0-2.6
10	3.0 2.6-3.3	3.3 3.0-3.9	4.0 3.5-4.5		3.0 2.5-3.5	3.4 2.9-3.7	2.9 2.4-3.4

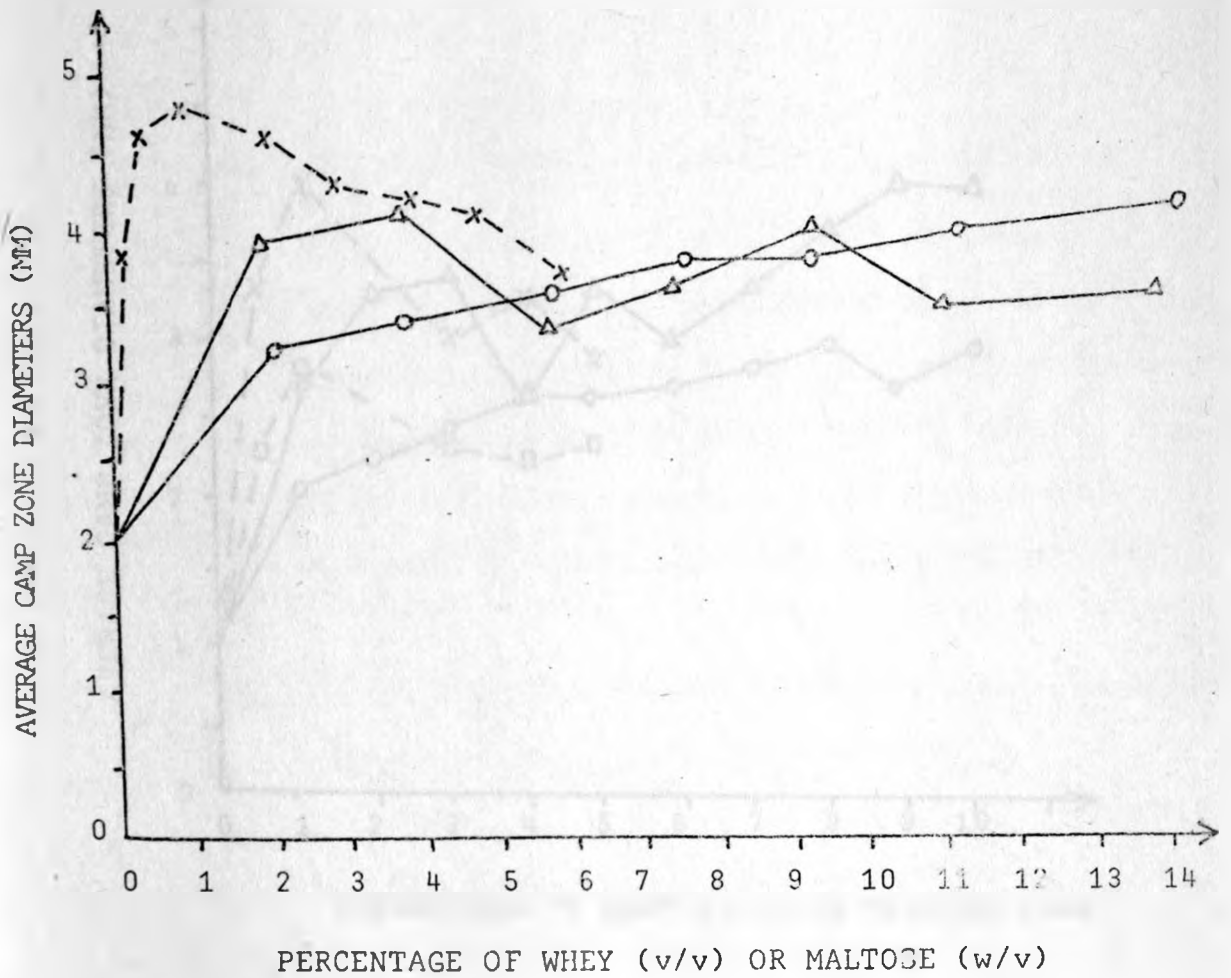(Column "2": plates not readable due to laboratory error)

Figure 1: Effect of whey (acid-produced and neutralized), maltose and 14% whey plus increasing concentration of maltose on CAMP reaction zones in the TKT-medium.

Δ = 1% maltose + increasing concentration of whey

O = Whey

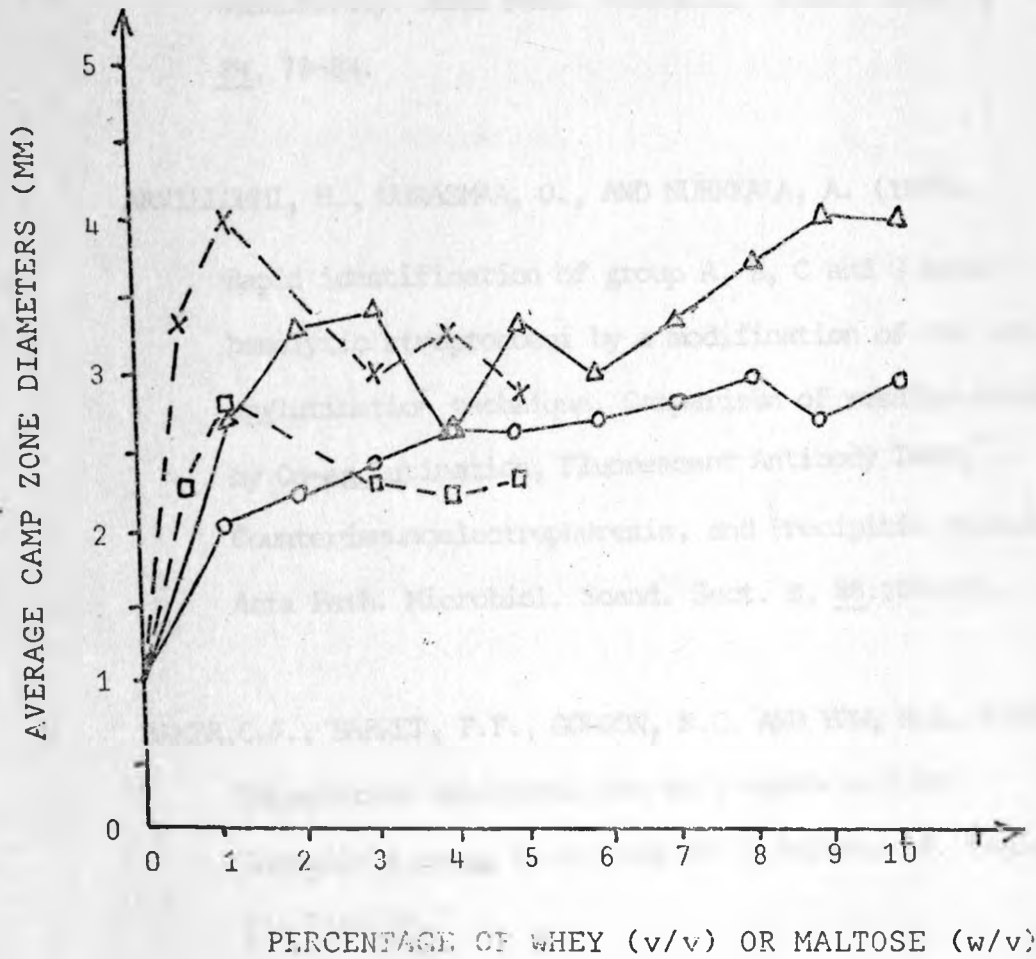X = 14% whey + increasing concentration of maltose

Figure 2: Effect of whey (rennin-produced), maltose, whey plus
          increasing concentration of maltose and maltose plus
          increasing concentration of whey on the CAMP reaction
          zones in the TKT-medium.

   ⊐ — Maltose

   O — Whey

   △ — 1% maltose + increasing concentration of whey

   X — 10% whey + increasing concentration of maltose

REFERENCES

ARVILLOMMI, H. (1976). Grouping of beta-hemolytic streptococci by using co-agglutination, precipitation or bacitracin sensitivity. Acta Path. Microbiol. Scand. Sect. B 84, 79-84.

ARVILLOMMI, H., UURASMAA, O., AND NURKKALA, A. (1978).

Rapid identification of group A, B, C and G beta-hemolytic streptococci by a modification of the co-agglutination technique. Comparison of results obtained by Co-agglutination, Fluorescent Antibody Test, Counterimmunoelectrophoresis, and Precipitin Technique. Acta Path. Microbiol. Scand. Sect. B, 86:107-111.

BAKER, C.J., BARRET, F.F., GORDON, R.C. AND YOW, M.D. (1973). Suppurative meningitis due to streptococci of Lancefield group B: a study of 33 infants. J. Pediatr. 82: 724-729.

BAKER, C.J., AND BARRET, F.F. (1974). Group B streptococcal infections in infants: The importance of the various serotypes. J. Am. Med. Assoc. 230:1158-1160.

BAKKEN, G. (1977). Personal communication.

BLATE, P. AND KJEMS, E. Personal communication. Cited by Christensen et al. (1973).

BLOBEL, H. (1977). Bacteriology of Bovine Mastitis. Dairy
    Sci. Abstr. 1977, Vol. 39 abstr. 4595.

BLOOD, D.C. (1978). The Control of Subclinical Mastitis.
    The Kenya Veterinarian, 2:3-5.

BRAMLEY, A. (1978). The Effect of Subclinical Staphylococcus
    epidermidis Infection on the Lactating Bovine Udder on
    its Susceptibility to Infection with Streptococcus
    agalactiae or Escherichia coli. Br. Vet. J. 134,146.

BROWN, J. FARNSWORTH, R., WANNAMAKER, L.W., AND JOHNSON, D.W.
    (1974). CAMP Factor of Group B Streptococci: Production,
    Assay and Neutralization by Sera from Immunized Rabbits
    and Experimentally Infected Cows. Infect. Immun. 9:377-383.

BUTTER, M.N.N. AND deMORE, C.E. (1967). Streptococcus agalactiae
    as cause of meningitis in the newborn, and bacteremia in
    adults. Differentiation of human and animal varieties.
    Antonie Van Leeuwenhoek; J. Microbiol. Serol. 33:439-450

CHRISTENSEN, P., KAHLMETER, G., JOHNSON, S. AND KRONVALL, G.
    (1973). New Method for Serological Grouping of Strepto-
    cocci with Specific Antibodies Adsorbed to Protein A-
    containing Staphylococci. Infect. Immun. 7:881-885.

CHRISTIE, R., ATKINS, N.E. AND MUNCH-PETERSEN, E. (1944).
A note on a lytic phenomenon shown by group B
streptococci. Austral. J.Exp. Biol. Med. Sci. 22,197-200.

CHRISTIE, R., AND GRAYDON, J.J. (1941). Austral. J. Exp. Biol.,
19, p 9. Cited by Christie et al., 1944.

CURTIS, S.N. AND KRAUSE, R.M. (1964). Antigenic Relationships
Between Group B and G Streptococci. J. Exp. Med.
120:629-637.

DARLING, C.L. (1975). Standardization and Evaluation of
the CAMP Reaction for the Prompt, Presumptive
Identification of Streptococcus aglactiae (Lancefield
Group B) in Clinical Material. J. Clin. Microbiol.
1:171-174.

DEIBEL, R.H. AND SEELEY JR., H.W. (9174). Bergey's Manual
of Determinative Bacteriology, Eighth Edition, The
Williams and Wilkins Company / Baltimore. p 490-509.

DOBBINS, C.N. (1970). Herd milking order: an idea saves
dairymen dollars. Mod. Vet. Pract. 51:41-43.

DOBBINS, C.N. (1977). Mastitis Losses. JAVMA 170,1129-1132.

DODD, F.H. (1971). Strategy of Mastitis Control. The Control
of Bovine Mastitis, The National Institute for
Research in Dairying, Shinfield, Reading, Berkshire
England. p. 7-13.

DODD, F.H., WESTGARTH, D.R. GRIFFIN, T.K. (1977).
Strategy of Mastitis Control. JAVMA 170, 1124-1128.

ENGBRETSEN, O. (1971). Group B Streptococci. The Application
of CAMP-Esculin Plate in the Diagnostic Routine.
Nord. Vet. Med. 23,419-426.

ESSEVELD, H., DANIELS-BOSMAN, M.S.M. AND LEIJNSE, B. (1958).
Some observations about the CAMP reaction and its
application to human β-hemolytic streptococci.
Antonie Van Leeuwenhoek, 24,145-156.

FACKLAM, R.R. (1974). Manual of Clinical Microbiology,
Second Edition. American Society for Microbiology
1913 I St., NW. Washington DC 20006. p. 96-108.

FALLON, R.J. (1974). The rapid recognition of Lancefield
group B hemolytic streptococci. J. Clin. Path.,
27, 902-905.

FORBES, D. (1970). The Pathogenic Significance of Various
Intramammary Infections. Br. Vet. J. 126, 260.

FRANCIOSI, R.A., KNOSTMAN, J.D. AND ZIMMERMAN, R.A. (1973).
Group B streptococcal neonatal and infant infections.
J. Pediatr. 82:707-718.

FRAZIER, W.C. (1967). Contamination of foods from natural
sources. Food Microbiology, Second Edition, Mcgraw-Hill
Book Company, p. 63-72.

GIESECKE, W.H. (1975). The defination of Bovine Mastitis
and the diagnosis of its subclinical types during
normal lactation. International Dairy Federation.
Proceedings on Seminar on Mastitis Control, 1975,
p. 62-70.

GHOROURY, A.A. (1950). Comparative studies of group B
streptococci of human and bovine origin: Serological
Characters. Am. J. Public Health. 40, 1278-1284

GREER, D.O. AND PEARSON, J.K.L. (1973). Streptococcus
agalactiae in Dairy Herds. Its Incidence and Relationship
to Cell Count and Inhibitory Substance Level in Bulk
Milk. Br. Vet. J. 129, 544.

GREER, D.O., POLLOCK, D.A. AND PEARSON, J.K.L. (1978).
A comparison between four serological methods used in
the identification of Str. agalactiae. Br. Vet. J.
134: 572-577.

HANSEN, A. WINTHER, O. (1953). On Employment of Staphylococcus
Toxin in Examination of Pooled Milk for Mastitis
Streptococci. Nord. Vet. Med. 5, 349-358.

HARN, G. AND NYBERG, I. (1976). Identification of streptococcal
groups A, B, and G by slide co-agglutination of
antibody sensitized protein A-containing staphylococci.
J. Clin. Microbiol. 7, 99-101.

HARRISON, J. (1941). Lancefield group B Streptococci on
the hands of milkers and others. J. Dairy Res. 12:18.

HAUGE, S. AND ELLINGSEN, J.K. (1953). Selective Agar Medium
(TKT-medium) for Demonstration of Group B Streptococci
in Samples of Producer Milk. Nord. Vet. Med. 5:539-547.

HALVEKA, B. (1944). Incidence of Streptococcus agalactiae
and Staphylococcus aureus on the udders and in milk
of cows. Dairy Sci. Abstr. 1976 Vol. 38 abstr. 4465.

HELMAN, C., HENTSCH, J. AND STALDER, B. (1971). Studies
on streptococci isolated from the milk of mastitis
and healthy cows, using the CAMP test and group
specific sera. Berl. Munch. Tierarztl. Wschr. 84:121-124.

HERBERT BRAUSTEIN, M.D., ELON B. TUCKER, M.A., AND CECIL
GIBSON. (1969). Identification and Significance of
    Streptococcus agalactiae (Lancefield Group B).
    AM. J. Clin. Path. 51:207-213.

HOUSE, J.A. AND BADAKHSH, F.F. (1975). The Role of Bacterial
    Culture in Bovine Mastitis Control. Practising
    Veterinarian 45:5-6.

HRYNIEWICZ, W., HECZKO, P.B., LUTTICKEN, R. AND WANNAMAKER,
L.W. (1976). Comparison of Three Methods for Grouping
    Streptococci. J. Clin. Microbiol. 4, 28-31.

HUGHES, D.L. (1953). Experiments on methods to control the
    spread of Streptococcus agalactiae in a dairy herd.
    Vet. Record, 65:1.

IVASHURA, A.I. (1972). CAMP Test as a reliable method of
    identifying streptococci of serological group B.
    Dairy Sci. Abstr. 1975, Vol. 37 abstr. 3627.

JAARTSVELD, F.H.J. (1963). The development and application
    of the Brabant Mastitis Reaction (BMR). International
    Mastitis Congress, Boxtel, the Netherlands, p. 43-46.

JACKSON, E.R. (1970). An Outbreak of Teat Sores in a
    Commercial Herd Possibly Associated with Milking
    Machine Faults. Vet. Record 87, 2-6.

JASPER, D.E AND DELLINGER, J.D. (1968). Use of Crude β-staphylococcal hemolysin for the presumptive recognition of Streptococcus agalactiae.AM. J. Vet. Clin. Path. 2:43-47.

JOHNSON, M.E., MARTIN, J.H., BAKER, R.H. AND PARSONS, J.G. (1977). A Comparison of Several Assay Procedures to Detect Penicillin Residues in Milk. J. Food Protection 40: 785-789.

JOKIPII , A.M.M. AND LIISA JOKIPII (1976). Presumptive identification and antibiotic susceptibility of group B streptococci. J. Clin. Path. 29:736-739.

KARAKAWA, W.W., WAGNER, J.E. AND PAZUR, J.H. (1971). Immunochemistry of the Cell-wall Carbohydrate of Group L Hemolytic Streptococci. J. Immun. 107, 554-562.

KARIUKI, D.P. (1977). The Use and Abuse of Antibiotics in Livestock. The Kenya Veterinarian, 1:8-9.

KARIUKI, D.P. (1978). Personal communication.

KENYA GOVERNMENT VETERINARY ANNUAL REPORTS, 1950-71.

KIRKBRIDE, C.N. (1975). Report of the Mastitis Committee. In Proceedings. US Anim. Health Associ., Portland, OR, 1975 (1976): 157-158.

KLEIN, L.A.,AND KLECKNER, A.L. (1941). Univ. Pa. Vet.

    Ext. Quart. 41, 13. Cited By Stableforth, 1959.

KRAUSE, R.M. (1963). Antigenic and biochemical composition

    of hemolytic streptococcal cell wall. Bact. Rev.

    27, 369.

KRONVALL, G. (1972). A rapid slide co-agglutination method

    for typing pneumococci by means of specific antibody

    adsorbed to protein A-containing staphylococci. J.

    Med. Microbiol. 6: 187-190.

LANCEFIELD, R.C. (1933). A serological Differentiation of

    Human and Other Groups of Hemolytic Streptococci. J.

    Exp. Med. 57, 571-595.

LAUERMAN, L.H., ORLIS, W.A., BUCK, H.A. AND LUTU, W.Z. (1973).

    Bovine Mastitis in Kenya. Bull. Epizoot. Dis. Afr.

    21: 167-170.

LINDE, C.G., HOLMBERG, O. AND ASTROM, G. (1975). An Attempt

    to Superimpose Staphylococcus aureus, Streptococcus

    agalactiae upon Staphylococcus epidermidis Infection

    in the Cow's Udder. International Dairy Federation.

    Proceedings of Seminar on Mastitis Control 1975 p. 391-394.

LINDQVIST, K. (1978). Personal communication.

LITTLE, R.B., BROWN, J.H., AND PLASTIRIGE, W.N. (1946a).
Bovine Mastitis, ed. by Little, R.B. and Plastrige,
W.N. New York; Mcgraw-Hill., p. 167.

LITTLE, R.B. AND PLASTIRIGE, W.N. (1946b). Cited by
Stableforth, 1959.

MADSEN, P.S., KLASTRUP, O., OLSEN, J.S., PENDERSEN, P.S.
(1976). Herd incidence of bovine Mastitis in four
Danish dairy districts. Relation between frequency of
mastitis and cell counts on bulk milk samples.
Nord. Vet. Med. 28: 100-107.

MAINA WANJIGI, (1972). Agriculture and Land Tenure in
Kenya. East Africa: Its peoples and resources,
Second Edition, edited by W.T.W. Morgan, p. 177-188.

MATTIC, A.T.R., SHATTOCK, P.M.F. AND MOREIRA, J. M. (1941).
The relationship of methods of bacteriological
examination to the eradication and control of
mastitis Streptococcus agalactiae. I. The use of
enriched technique in revealing streptococci
infection of the cow's udder. II. Streptococcus
agalactiae infections in heifers. J. Dairy Res.
12, 139-154.

MCDONALD, J.S., MCDONALD, T.J. AND ANDERSON, A.J. (1975).
Characterization of and Bovine Intramammary Infection
by Group B Streptococcus agalactiae of Human Origin.
In proceedings. US Anim. Health Assoc. Portland, Or,
1975 (1976) 150-156.

MCDONALD, J.S. (1977). Streptococcal and Staphylococcal
Mastitis. JAVMA 170: 1157-1159.

McCARTY, M. (1958). Cited by Wilson, G.S. and Miles, A.A.
1975.

MCLEOD, D.H. AND WILSON, S.M. (1951). Milk yield in
relation to infection with Str. agalactiae. J. Dairy
Res. 18: 235-239.

MERCHANT, I.A., AND PACKER, R.A. (1971). Veterinary
Bacteirology and Virology. 7th Edition, Iowa State
University Press, Ames, Iowa. p. 211-236.

MUNCH-PETERSEN, E. AND CHRISTIE, R. (1947). On the effect
of the interaction of staphylococcal betatoxin and
Group B streptococcal substance on red blood corpuscles
and its use as a test for identification of Strepto-
coccus agalactiae. J. Path. Bact. 59, 367-371.

MUNCH-PETERSEN, E., CHRISTIE, R. AND SIMONS, R.T. (1945).
Austral. J. Exp. Biol. and Med. Sci., 23, 193.
Cited by Murphy, Stuart and Reed, 1952.

MURPHY, J.M., STUART, O.M. AND REED, F.I. (1952). An
    evaluation of the CAMP test for the identification
    of Streptococcus agalactiae in routine Mastitis
    Testing. Cornell Vet. 42: 133-147.

MURPHY, J.M., STUART, O.M. AND REED, F.I. (1947). Cited
    by Murphy, Stuart and Reed, 1952.

NATIONAL MASTITIS COUNCIL, (1969). Microbiological Pro-
    cedures for the Diagnosis of Bovine Mastitis. 910
    Seventeenth Street, N.W. Washington D.C.

NEAVE, F.K., DODD, F.H. KINGWILL, R.G. AND WESTGARTH, D.R.
    (1969). Control of Mastitis in the Dairy Herd by
    Hygiene and Management. J. Dairy Sci. 52, 696-707.

NEAVE, F.K. AND JACKSON, E.F (1971). The prevention of
    intramammary infection. The Control of Bovine
    Mastitis, The National Institute for Research in
    Dairying, Shinfield, Reading, Berkshire, England
    p. 15-24.

NEWBOULD, F.H.S. AND NEAVE, F.K. (1965). The response of
    bovine mammary gland to an infusion of staphylococci.
    J. Dairy Res. 32: 163.

NEWBOULD, F.H.S. (1975). The Possibility of Eradicating
Specific Infections in Mastitis Control. International
Dairy Federation. Proceedings of Seminar on Mastitis
Control 1975: 382-388.

NARCROSS, N.L., OLIVER, N (1976). The distribution and
characterization of group B streptococci in New York
State. Cornell Vet. 66, 240-248.

OXOID MANUAL OF CULTURE MEDIA, Ingredients and other
laboratory services, 3rd Ed. 1973. Oxoid Ltd. London.

PATTERSON, M.J. AND HAFEEZ, A.B. (1976). Group B streptococci
in Human Disease. Bact. Rev. 40: 774-792.

PEARSON, J.K.L. (1971). Monitoring Control System-Evaluation
of Methods. Control of Bovine Mastitis, The National
Institute for Research in Dairying, Shinfield, Reading
Berkshire, England p. 93-104.

PEARSON, J.K.L., GREER, D.O. AND POLLOCK, D.A. (1976).
Streptococcus agalactiae in the smaller herd. Its
incidence in relation to somatic cell counts. Br. Vet.
J. 132: 588-594

PHILPOT, W.N. (1975). Prevention of Infection - Hygiene.
International Dairy Federation. Proceedings of
Seminar on Mastitis Control 1975. p. 155-164.

POSTLE, D.S. (1968). Evaluation of a selective medium
for screening bulk milk samples for Streptococcus
agalactiae. Am. J. Vet. Res. 29: 669-678.

POSTLE, D.S., NATZKE, R.P., EVEREST, R.W. (1971). Relation-
ship between leucocyte count in bulk milk and
apparent quarter infections in dairy herds. J. Milk
Fd. Technol. 34: 517-520.

PRAKASH, K., RAVINDRAN, AND SHARMA, K.H. (1973). Increasing
incidence of group B betahemolytic streptococci from
human sources. Indian J. Med. Res. 61: 506-513.

PRESCOTT, S.C. AND BREED, R.S. (1910). The determination of
the number of body cells in milk by a direct method
J. Inf. Dis. 7:632. Cited by Postle 1968.

REITER, B. AND ORAM, J.D. (1967). Bacterial Inhibitors in
Milk and Other Biological Fluids. Nature 216, 328-330.

RENNER, E. (1975). Investigation of Some Parameters of the
Milk for the Detection of Subclinical Mastitis.
International Dairy Federation. Proceedings of Seminar
on Mastitis Control 1975. p. 53-58.

RICHARD ROSNER (1977). Laboratory Evaluation of a Rapid
    Four-Hour Serological Grouping A, B, C and
    G Beta-Streptococci by the Phadebact Streptococcus
    Test. J. Clin. Microbiol. 6: 23-26.

ROBINSON, J.A. AND MEYER, F.P., (1966). J. Bact. 84, 163.
    Cited by Wilson and Miles, 1975.

SAXEGAARD, F. (1977). Comparative grouping of mastitis
    streptococci by means of co-agglutination and
    precipitation. Acta. Vet. Scand. 18, 509-514.

SCHALM, O.W. (1942). Streptococcus agalactiae in the udder
    of heifers at parturation traced to suckling among
    calves. Corneli Vet. 32: 49.

SCHALM, O.W. AND NOORLANDER, D.O. (1957). Experiments and
    Observations Leading to Development of the California
    Mastitis Test. JAVMA 130: 199-207.

SCHALM, O.W., CARROL, E.J. AND JAIN, N.C. (1971). Bovine
    Mastitis. Lea and Febiger, Philadelphia pg. 94-208.

SCHALM, O.W. (1977). Pathologic changes in the Milk and
    Udder of cows with Mastitis. JAVMA 170: 1137-1140.

SCHULTZ, E. (1972). Comparison of the direct sediment plating methods for the detection of Streptococcus agalactiae and Staphylococcus aureus in quarter milk samples. Dairy Sci. Abstr. 1976, Vol. 38 abstr. 1759.

SCHMIDT, W.C. (1952). J. exp. med. Wschr., 50, 849-909. Cited by Wilson and Miles, 1975.

SHUMAN, R.D., NORD, R.W. BROWN AND WESSMAN, (1977). Biochemical and Serological Characteristics of Lancefield Group E, P and U streptococci and Streptococcus uberis. Cornell Vet. 62: 540-568.

SIMMONS, R.T. AND KEOGH, E.V. (1940). Physiological characters and serological types of haemolytic streptococci of groups B, C and G from human sources. Austral. J. Exp. Biol. Med. Sci. 18: 151-161 cited by Patterson, J.M. and Hafeez, A.E.B., 1976.

SMITH, A.R. AND JOHNSON, S.M. (1972). Rapid Diagnosis of streptococcus agalactiae and Streptococcus uberis. J. Milk Fd. Technol., 35: 540-568.

STABLEFORTH, A.W. (1959). Infectious Diseases of Animals, Diseases Due To Bacteria, Volume 2. Academic Press, Inc., New York, p. 589-650.

STEVEN,M. GUBASH (1978). Synergistic Hemolysis Phenomenon
    Shown by an Alpha-Toxin-Producing Clostridium
    perfringens and Streptococcal CAMP Factor in
    Presumptive Streptococcal Grouping. J. Clin.
    Microbiol. 8: 480-488.

THAL, E. AND OBIGER, G. (1969). The CAMP phenomenon of
    Streptococcus agalactiae and the new serological'
    streptococcus group "U" and other types of bacteria.
    Berl. Munch. Tierarztl. Wschr. 82, 126-130.

TEBBUTT, G.M., COLEMAN, D.J. AND MCGHIE, D. (1976).
    Grouping of betahemolytic streptococci with group
    specific antibodies adsorbed to staphylococcal protein
    A.   J. Clin. Path. 29, 1085-1087.

TOLLE, A. (1975). Mastitis - the Disease in Relation to
    Control Methods. International Dairy Federation
    Proceedings of Seminar on Mastitis Control 1975, p. 3-15.

WARD, D.G. (1978). Personal communication.

WEITZ, B.G.F. (1971). The Control of Bovine Mastitis, the
    National Institute for Research in Dairying, Shinfield
    Reading, Berkshire, England. p. 1-6.

WEISNER, H.U., HUBLER, C. (1975). Quality Requirements for
    Raw Milk - Absence of Group B streptococci. Dairy Sci.
    Abstr. 1975, Vol. 37 abstr. 3376.

WILKINSON, H.W. (1977). CAMP-Disk Test for Presumptive
Identification of Group B Streptococci. J. Clin.
Microbiol. 6: 42-45.

WILSON, G.S. AND MILES, A.A. (1975). Principles of
Bacteriology, Virology and Immunity. 6th Edition,
Edward Arnold (Publishers) Ltd., 25 Hill Street,
London WIX 8LL. Vol. 1 p. 712-745.

WILSON, C.D. AND SLAVIN, G. (1950). A direct culture
method for examination of milk samples. J. Comp.
Path. 60, 230-234.

WRIGHT, C.L. (1977). The significance of cells in bovine
bulked milk supplies. Vet. Record 100: 8-9.

ZLOTNICK, I. (1947). J. Comp. Path. 57, 196. cited by
Wright, 1977.

Appendix I: Sampling areas and distribution of the
Kenya Co-operative Creameries.