Tijdschrift voor diergeneeskunde

Provinciale Gezondheidsdienst voor Dieren in Noord-Brabant
Tijdschrift voor Diergeneeskunde, Vol. 89, 1964 supplementum II.

International Mastitis Congress

25-27 September 1963

Proceedings

Tijdschrift voor Diergeneeskunde, Vol. 89, 1964 supplementum II.

TIJDSCHRIFT VOOR DIERGENEESKUNDE

PUBLISHED BY THE ROYAL NETHERLANDS VETERINARY ASSOCIATION

Published on the 1st and 15th of each month

SUBSCRIPTION: *f* 40,—, ABROAD: *f* 45,— YEARLY, PAYABLE IN ADVANCE. POSTAL CHEQUE ACCOUNT NO. 511606 IN THE NAME OF KONINKLIJKE NEDERLANDSE MAATSCHAPPIJ VOOR DIERGENEESKUNDE

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Opening of the Conference

Welkomstwoord.

door J. VAN DOREN*)

Dames en Heren, geachte aanwezigen,

Namens het Bestuur van de Provinciale Gezondheidsdienst voor Dieren in Noord-Brabant heet ik U hedenmorgen van harte welkom.
Wij, het Bestuur van de Provinciale Gezondheidsdienst voor Dieren in Noord-Brabant, stellen het bijzonder op prijs dat U in zo'n grote getale gehoor hebt willen geven aan onze uitnodiging om dit mastitiscongres, op internationaal niveau, te komen bijwonen.
Tezamen kunnen we nu van gedachten wisselen omtrent deze dierziektebestrijding van de toekomst.

Het spijt me dat de scholing, die ik in mijn jeugd heb meegemaakt, niet van dien aard is, dat ik hier in de diverse talen van de landen waarvan hier vertegenwoordigers aanwezig zijn, een welkom kan toespreken. De schuld hiervan is bij mijzelf gelegen, de last hiervan zal ik dan ook door dit leven moeten dragen. Ik zou dan ook Dr. B r u s, die straks het congres zal openen, willen vragen, om ook in een andere taal, b.v. de Engelse taal, ik leef n.l. niet in de veronderstelling dat de heer Brus ook de scandinavische talen stuk voor stuk onder de knie gekregen heeft, deze woorden in het kort te vertalen, opdat het welkom dat ik U van ganser harte toeroep namens het Bestuur ook als zodanig moge klinken in voor U allen begrijpelijke woorden.

Het mastitis-vraagstuk waarvoor wij deze dagen op het congres gesteld worden laat ons, als praktische veehouders, niet onberoerd.
Wij hebben in de loop der jaren, wat deze ziekte betreft, alle mogelijke narigheden ondervonden. Wij kennen moeilijkheden bij de melkwinning, bij de melkbehandeling en ook bij het houden van vee als zodanig, alle zaken die stuk voor stuk van invloed zijn op de portemonnaie van de veehouder. Vandaar dan ook dat 't vanzelfsprekend is, dat hetgeen in de naoorlogse jaren op dit terrein door U aan onderzoek is verricht, dank verdient.
Wij zijn U allen daarvoor erkentelijk.

Wanneer wij dan hier, voor wat betreft de Gezondheidsdienst voor Dieren in Noord-Brabant, er in geslaagd zijn — ons richtende op het onderzoek op de ontstekingscellen welke in de melk aanwezig kunnen zijn — een bruikbare methode te vinden, dan verheugen wij ons erop U deze manier van onderzoek te kunnen demonstreren. Mogelijk kan deze methode, door er een beetje aan te schaven, gericht worden op de praktijk van de zuivel en op de dagelijkse gang van zaken op de boerderij. Een bruikbare en niet

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*) J. van Doren; vice-voorzitter van de Provinciale Gezondheidsdienst voor Dieren in Noord-Brabant; Rechterstraat 80, Boxtel.

kostbare methode voor het aantonen van deze ontstekingscellen in de melk zou een basis kunnen vormen voor de bestrijding van de mastitis als zodanig.

Wanneer wij hier hedenmorgen dan theoretische beschouwingen zullen aanhoren betreffende het mastitis-vraagstuk in z'n diverse vormen, wanneer in de namiddag demonstraties zullen volgen op het laboratorium en deze zullen worden toegelicht door mensen uit de praktijk zowel van Gezondheidsdienst als uit de zuivelindustrie, zijn wij daarover verheugd.
Wanneer wij dan morgen, zowel op de boerderij als op de zuivelfabriek, praktijkdemonstraties krijgen, dan hoop ik, dat hetgeen zowel vandaag als morgen hier gegeven zal worden, voor U aanleiding zal vormen, U intens op de problemen te werpen en in de discussie het zover te brengen dat dit mastitiscongres vruchten mag afwerpen.

Ik hoop dat deze samenkomst datgene voor U allen zal brengen wat U ervan verwacht en dat het voor ons als veehouders zal mogen geven, in de naaste toekomst, een voor de praktijk bruikbare onderzoekmethode en bestrijdingsvorm die de nadelen van deze uierontstekingen zoveel mogelijk zullen gaan voorkomen en beperken.

Nogmaals welkom. Ik dank U zeer.

Opening of the Congress.

by D. H. J. BRUS*)

Ladies and Gentlemen,

You were addressed by Mr. J. van Doren, vice-president of the Board of the Animal Health Service in the province of North Brabant. He regretted not being able to welcome you in your own language. Mr. van Doren is a live-stock owner and a leading figure in various organisations. He is also chairman of the Board of the Co-operative Dairy Works „Campina" which we shall visit tomorrow.
The chairman of our committee, Dr. C. J. van Meel, died two months ago. He was extremely interested in everything concerned with the stamping out of animal diseases. In the parliament of our country, he championed the cause of live-stock owners.
During his last weeks of life in hospital, he spoke of this congress and we consider it a matter of great regret that it was not given to him to be here to-day.

Ladies and Gentlemen, it was in February 1963 that we started to contemplate a mastitis congress. At that time, we received a letter from Dr. Schalm, in which he wrote that he was planning to visit Europe in the autumn of that year.
At the same time, a number of foreign visitors from the Scandinavian countries and Germany were our guests in Boxtel. As we discussed problems relating to the diagnosis and treatment of mastitis, it became apparent that it would be desirable to develop methods of investigation and treatment as similar to one another as possible in various countries.
The stamping out of tuberculosis of cattle and brucellosis has almost been completed in the north-west part of Europe.
The Scandinavian countries were the first to conclude their eradication programme. They also were the first to study the possibilities of mass control of mastitis. The methods adopted in the stamping out of tuberculosis and brucellosis, consisting in eradication of the bacteria causing these diseases, are not suited for mass control of mastitis.

The question may be asked whether the pure "microbe-hunter" will be the front-line soldier in this field. Investigators in the various countries do not speak the same language; this is true both in the literal and in the figurative sense of the word but it also applies to the different methods of investigation. It therefore is not easy to understand one another. We hope that this congress will help investigators to understand each other in the fields of diagnosis and treatment of mastitis or, better still, in that of the prevention of bovine mastitis.

I therefore am glad to be in a position to welcome all of you here in North Brabant and I shall start with those guests who have come from afar.
I am very glad to see you here, Dr. Schalm. As I said, the news of your coming to Europe marked the beginning of our plans for the con-

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gress. Your method of identifying cells in the milk rather than bacteria made it possible to carry out mass investigations. To start an organized campaign against a disease, methods of mass investigation are essential and these methods should be simple, reliable and inexpensive.

Dr. Funke of Sweden, heartily welcome to North Brabant.
Norway is represented here by Dr. A. Bratlie, Dr. A. Kyrkjebö and Mr. Ivar Engan Skei. Welcome to our country.
We have always regarded Denmark as an example of tuberculosis and brucellosis control. It was Dr. Livoni of Denmark, who made efforts to diagnose and cure mastitis in the field. I regret that he was not able to attend and therefore I am doubly glad to see you here, Dr. Klastrup and Dr. Mallin Olsen.
Dr. Hämmäinen, your country, Finland, has always enjoyed a reputation of sympathy in the Netherlands. A small country with great people.

For Great Britain, Dr. Edwards and Mr. Kingwill are attending this congress. I am sure that there are good contacts between investigators of your country and those of countries in the north-west part of the continent. I hope that there will be more contacts in the fields of practical organization and control of animal diseases.

From Belgium, we have Prof. Vanderplassche of the State University of Ghent. The co-operation between the Sterility Committee of your university and workers in this field in The Netherlands is not a sterile one. I am glad that you are willing to help us by conducting discussions to-day.
Dr. Florent of the State Serum Institute in Ukkel, welcome here. Dr. Vereertbruggen and Dr. Tuyttens also are guests from Belgium. Dr. Tuyttens, I regard you as a representative of the very young Health Services in Belgium. We have been in very close contact with you and some of your fellow-workers in our laboratory. I am sure that we shall work together in the future.

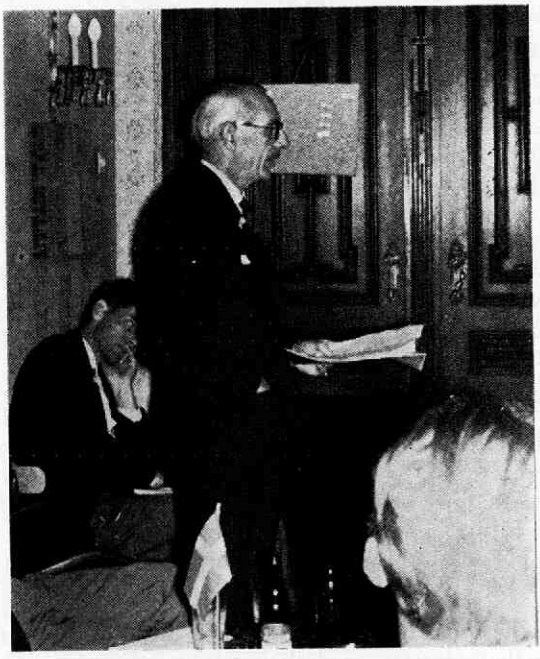
A country in which work on mastitis has been done for a long time and in many organizations, is Germany.
Es freut mich sehr, dass Sie heute in so grosser Zahl hier sind.
Von den deutschen Tiergesundheitsämtern darf ich begrüssen: Dr. Tilgner aus Kiel, Dr. Mempel aus Münster, Dr. Richter aus München, Dr. Scheiner aus Hannover. Dr. Schiel aus Oldenburg hat auch eingeschrieben, ist aber leider durch Krankheit verhindert. Es freut mich Sie, Dr. Plöge, an Stelle Dr. Schiels begrüssen zu können. Sie, Dr. Tilgner, sind bereit als Diskussionsleiter aufzutreten.
Von den Staatlichen Tierärztlichen Untersuchungsämtern und Universitäten Deutschlands sind unsere Gäste: Dr. Kauker aus Kassel, Dr. Kielwein aus Aulendorf, Dr. Kraus aus Hannover, Prof. Dédié aus Aulendorf und Dr. Zettel aus Kassel.
Es freut mich sehr, zwei junge Gelehrten in unserer Mitte zu haben, Fräulein Herrguth und Herrn Köster, die ihre Doktorarbeit auf dem Gebiete der Mastitis machen wollen. Wir sind sehr darauf gespannt, was ihre Forschungen uns bringen werden.
Fräulein Weith und Fräulein Herrguth heisse ich besonders herz-

lich wilkommen. Sie sind die weiblichen Stimmen in unserem Chor. Ich bin davon überzeugt, dass sie leise aber hell ertönen werden.

And now I come to the guests from our country, The Netherlands.
The members of our Board and of our own Mastitis Committee are at home here. This also is true of the directors and bacteriologists of the other Provincial Health Services.
A special word of welcome to the representative of the Ministry of Agriculture and Fisheries, Mr. V e r v o o r n.
From the Central Veterinary Research Institute, our fellow veterinarian V a n D i j k will be present during these days.
From the Faculty of Veterinary Medicine of the State University of Utrecht, we see Mr. N o o i t g e d a g t and, from the Agricultural College of Wageningen, Dr. S c h i p p e r.
Of the members of the Central Mastitis Committee, Mr. M o l, Director of the Milk Inspection Station, Amsterdam, is attending the congress.
Of the Higher Dairy School, we see Mr. K r o m w i j k here.
Mr. K e r k h o f and Mr. P e i j n e n b u r g are members of the Regional Milk Hygiene Organs. We have been working together for the past two years. I feel sure that your work and ours will be on a common basis in the future. I am very grateful to your Foundation for the material received for this congress.
I should like to say the same to Mr. B a n n e n b e r g of the Co-operative Dairy Works "Campina".
Mr. d e R o o ij of the Dairy Research Institute. We have been working together for two years in order to determine the relationship between bovine mastitis and the management of cattle by live-stock owners to the best of our ability. The initial results are encouraging but more investigations will have to be carried out.
A special word of welcome for Mr. K a r s e m e i j e r of the Royal Netherlands Veterinary Association. Veterinary practitioners and the Health Services are successfully co-operating in tuberculosis and brucellosis control. I am sure that we shall need one another in the control of other diseases in the future.
And, last but not least, I welcome the secretary of the Animal Health Committee, Dr. Z u i j d a m.

I hope to find the opportunity of addressing a special word to the various speakers when they start to read their papers.
I shall conclude by expressing the wish that anyone who speaks, may do so slowly and will say very wise things in simple words such as those which you normally use when you are a child of about twelve. It is not easy to speak a foreign language correctly. I should say that, in this case, it is more important to speak clearly than to speak correctly. I hope that we may have a successful congress but also that we will have pleasant days.

I declare the congress open.



Discussion during the congres.

Lectures and Discussions

General introduction concerning Mastitis and Mastitis-diagnosis.

by A. VAN DER SCHAAF*)

Introduction.

Dr. Brus: *May I introduce to you Prof. A. van der Schaaf, professor in bacteriology of the Veterinary Faculty of the State University of Utrecht.*

After he got his degree he worked at the Institute of Preventive Medical Science in Leyden. Later he was in Indonesia at the State Serum Institute in Bogor and also professor of the Veterinary School.
After the war he became a bacteriologist at the Animal Health Service in Friesland, the oldest service in The Netherlands. Since 1955 he is professor in Utrecht.

He was the promotor of the thesis by Dr. Jaartsveld on the Brabant Mastitis Reaction.
I am glad, Prof. van der Schaaf, to have the opportunity to ask you to speak.

Bovine tuberculosis in The Netherlands and most neighbouring countries has already been eradicated and, in addition, the anti-brucellosis campaign will be completed in the near future. Therefore the period that veterinary health services shall have time to occupy themselves with mastitis as a herd problem is rapidly approaching.
Mastitis causes considerable losses to farmers. This is not a new situation but this disease does seem to occur more frequently than formerly notwithstanding the fact that the arsenal of therapeutic drugs has grown enormously.
Mastitis as a herdproblem is the subject of this symposium and we are here to learn from each other about the extent of the problem, about the diagnostic laboratory methods now available and thirdly about the most promising therapeutic and preventive measures.
According to Little and Plastridge, bovine mastitis is an inflammation of the mammary gland which is characterized by tissue changes, which lead to changes in the secretory product of the tissue, whether the etiology be of an infectious, chemical, or thermal nature or result from an injury. Of those four causal factors the first and the last one are the most considerable, for chemical substances and overheating or chilling can very seldom be considered as important etiological factors. Concerning the infectious agents and injuries perhaps most veterinarians think that for the aetiology of mastitis in milkcattle germs are of much more importance than injurious causes.
Recent investigations of Stuart and coworkers in the Central Veterinary Laboratory at Weybridge indicate that the *Mycoplasma bovigenitalium* also has to be considered as a primary cause of mastitis. This agent

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is not filtrable, but as with viruses, it is very difficult to show its presence by microscopical and cultural methods and we do not know how frequently it occurs in The Netherlands. Nevertheless doubts have to be raised about Streptococci, Staphylococci, *Corynebacterium pyogenes, E. coli, Klebsiella* and *Pseudomonas aeruginosa* as primary causes of inflammatory conditions in the cow's udder.

Injuries and perhaps viruses come first as etiological factors. When these factors have done their job as pathfinders the different microbes, which can be considered as more or less ubiquitary, can invade the mucous membrane of the duct system and later on also the alveolar tissue.

It is of course not necessary that the injuries are of a violent character. On the contrary the slow acting and insidious momentums of disfunctioning milking machines and handmilkers are of more importance. Predisposing hereditary factors are also to be considered. There are for example heavy milking, long teats, pendulant udder, inequal lactation of hind and front-quarters and hormonal disbalance. Environmental factors as faulty construction of barns, short and narrow stalls, lack of bedding and a warm, humid atmosphere can be predisposing as well as for injuries as for bacterial infections. It will always be very difficult to unravel which is the real primary factor which causes the mastitis.

In agreement with the supposition that injuries form the primary causes of mastitis are the many subclinical cases of so called traumatic mastitis with more than 1 million leucocytes in 1 milliliter milk but without the wellknown pathogenic microbes.

This does not mean that bacteria have no significance for the pathogenesis of mastitis. On the contrary the bacteria are secondary invaders of the damaged tissue. They can affect the wounded or inflamed mucous membranes of the streak canal, the teat cistern or lactiferous sinus so seriously that an entire disfunction of the milkproducting acini follows.

The germs which can multiply in milk and form exo- or endotoxins are of course of most importance for a control program, but it would be a blunder when the combat against bovine mastitis would start with the systematic application of antibacterial therapeutics. The primary task will be to discover the herds with a mastitis-problem and then to find out which injuries predispose for subsequent infections by streptococci, the staphylococci and eventually yet other germs such as PPLO's.

Dr. J a a r t s v e l d has done a good job in studying the methods which would be useful to apply as screen tests and which have to indicate the problem herds. He made the conclusion that the Schalm- or Californian Mastitis Test, the C.M.T., was the most reliable one, and finally he modified the test from a plate test to a tube test. He completed the possibility for mass-application of the test by introducing an objective method for measuring the increase of the viscosity of mastitis milks caused by adding a 2% solution of sodium laurylsulphate. He then designed a capillary-tube of a fixed length and diameter which was fused to a funnel and showed that a flowtime through the capillary in more than 10 seconds of a quantity of 0,6 ml milk mixed with 0,4 ml reagent was an indication that the sample contained more than 500.000 cells per ml. For cattle in full lactation this may be considered as an indication of mastitis. In problem herds there are of course more than 1 cow with mastitis and so, as with the ring-

test in brucellosis, the viscosity-test can be used for screening samples of canmilk.
I had the honour and the pleasure to be promotor of Dr. J a a r t s v e l d and as godfather to the child of his brain I gave the test the name of Brabantse Mastitis Reactie or B.M.R.
A godfather usually gets also the possibility to contribute to a prosperous development of his godchild and therefore with the help of Mrs. K r a m e r, my technical assistant, we attempted some trials for improvement. We had no success with our attempts to conserve the milks in such a way that the tests could be postponed for 24 or 48 hours after sampling.
But is was shown that with milk samples stored at 6° Celcius the addition of sodiumhydroxide to the reagent in such a quantity (0,6 ml 10% NaOH per 40 ml 2% sodiumlaurylsulphate) that the pH rose to 12, the results of the test were the same on the first as on the second day after collecting the samples.
We also paid attention to the further standardisation of the test and we could demonstrate that is was not correct to carry out the B.M.R. when the milk and the reagent were cold. After storing in a refrigator the samples can be put in the incubator for about half an hour and then the reagent with a temperature between 20 and 30° C. can be added. If the milk or the reagent are too cold the intensity of the viscosity is diminished. This would result in wrong readings.
Pending these trials it could also be demonstrated that with samples which contained more than 1 million of cells per milliliter the flowtime was not exactly the same as when the test was repeated. The differences in results are caused by slimy clumps of leucocytes which can obliterate the capillary tube from time to time. These clumps can be invisible to the naked eye. When the number of leucocytes is less than 500.000 the differences in flowtime are negligible. For practical circumstances this means that a sample which gives three dots in the administration of the flowtime occasionally can also get four dots. The next tables illustrate all those observations in detail.
For the routine of the screening of thousands of milksamples, received daily from the dairies, these differences in flowtime are of no practical significance.

Influence of temperature during storing and testing on the result of the B.M.R.

Milksample stored at ± 20° C. during 20 hours*)

		Temperature of milk and reagent before mixing.			
		35° C.	25° C.	15° C.	5° C.
Flowtime in seconds	1st	18	17	19	11
„ „ „	2nd	17	29	13	11
„ „ „	3rd	14	19	13	5

Milksample stored at ± 6° C. during 20 hours*)

		Temperature of milk and reagent before mixing.			
		35° C.	25° C.	15° C.	5° C.
Flowtime in seconds	1st	78	46	52	31
„ „ „	2nd	40	87	33	19
„ „ „	3rd	25	21	18	10

*) See page 24.

It is my opinion that the B.M.R. can be very useful for those animal health services and milkcontrolstations which have to assist the farmers community to increase the quantity and improve the quality of their milk without extension of labour and costs. I hope that this symposium will introduce new ways for the anti-mastitis campaigns which would serve this purpose.

SAMENVATTING.

Nu de rundertuberculose in verscheidene landen is uitgeroeid en dit ook voor abortus-Bang praktisch het geval is, begint de tijd te naderen, dat meer aandacht besteed kan worden aan de mastitis als koppelziekte. Men krijgt de indruk, dat, hoewel de therapeutische mogelijkheden groter zijn geworden, het aantal probleembedrijven toch stijgt.

Bij de aetiologie van deze ziekte moet men denken aan verwondingen, infecties en verder aan chemische en thermische invloeden. De beide eerste zullen de belangrijkste zijn. Als infectieuze oorzaken dient men, naast bacteriën, ook aan *Mycoplasma bovigenitalium* te denken.

Spreker wijst er op, dat zelfs ontstoken kwartieren die melk met meer dan een miljoen ontstekingscellen per cm^3 bevatten, bacteriologisch steriel kunnen zijn. Men moet bij mastitis, als probleem, denken aan verwondingen en mogelijk ook aan virussen als primaire oorzaken. Bacteriën spelen meer een rol als parasieten, die nadien het verzwakte weefsel binnendringen. De bacteriën dient men als gelegenheidsparasieten en niet als primaire oorzaken te beschouwen.

De verwondingen behoeven maar uiterst klein te zijn. De verwondingen zijn niet alleen de directe oorzaken van uierontsteking, maar ook de toestand van het uier of de bouw van uier en tepels of de verhouding tussen voor- en achterkwartieren enz. kunnen een rol spelen. De bouw en het klimaat van de stal kunnen ook belangrijk zijn. Het starten van een georganiseerde bestrijding van mastitis als stalprobleem op basis van behandelingen met antibiotica, zou spreker een blunder vinden. Men moet op deze bedrijven de diepere oorzaken waardoor vermindering van weerstand in het uier optreden, gaan opzoeken en deze zoveel mogelijk wegnemen. Het zoeken naar en het behandelen van de begeleidende bacteriën is van aanvullende betekenis.

De Brabantse Mastitis Reactie is een geschikte methode om, via onderzoek van busmonsters, de probleembedrijven op te zoeken. Het is gebleken, dat, bij oelhoudende melkmonsters, de doorstroomtijd met het ouder worden van de melkmonsters terugloopt.

Tezamen met Mevr. K r a m e r heeft spreker gevonden, dat indien de testvloeistof op Ph 12 wordt gebracht dit effect grotendeels geneutraliseerd kan worden. Verder blijkt dat de B.M.R. het best bij een temperatuur van 20 à 30° C kan geschieden. Bij lagere temperaturen wordt de behandelde melk minder visceus. Bij melk met vrij veel cellen kunnen de doorstroomcapillairen wel eens door klompjes slijm verstopt raken, waardoor een te hoog aantal cellen wordt aangegeven.

Spreker vindt de B.M.R. een bruikbare methode voor Gezondheidsdiensten en Melkcontrole-stations. Deze toch moeten de veehouders helpen een zo goed mogelijke melk tegen een zo laag mogelijke kostprijs te produceren.

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*) The fresh milksample with 1.900.000 cells per ml. gave a variation in the B.M.R.-flowtime through the same capillary tube as follows in seconds recorded: 62, 32, 35, 36, 46, 70, 54, 50, 43.
It is divided in two parts. One half is put on the laboratory table (temperature ± 20° C.) and the other in the refrigerator. Both have been thoroughly mixed and thereafter pipetted in agglutination tubes in the quantities of 0,6 ml.
The alkaline reagent (pH 12) is pipetted in quantities of 0,4 ml. Three of each are put in waterbaths with a temperature of 35°, 25°, 15° and 5° C. during 15 minutes before mixing and subsequently recording of flowtimes.

Pathogenesis of Coliform Mastitis (Aerobacter aerogenes).

by O. W. SCHALM*) ; U.S.A.

Introduction.

Dr. B r u s: *Dr. Schalm; As I told you today, your coming to Europe was the inspiration for this congress.*
I am glad we already had a little talk in Hannover. I am sure that the mastitis problems in the U.S.A. and specially in California with the big dairy-herds can differ from the problems in some parts of Europe.
While we talk about mastitis and too many cells in milk you are already going a step further and thinking about the risks of too few cells in the milk.
We are looking forward what you are going to tell us about that problem.

It may seem that my lecture does not fit into the general trend of this conference but I am of the opinion that our experience with coliform mastitis may be of special interest to you. This form of mastitis could become a greater problem in the future when the more common forms of mastitis have been brought under control.
In the U.S.A. a number of dairy herds located at Universities and research centres have been subjected to rigid programs of control of streptococcal and staphylococcal mammary infections. These control programs have frequently employed extensive treatment of the infected mammary glands with antibiotic and therapeutic agents. It seems rather strange that at least six of such herds have been reported to have experienced considerable trouble from acute coliform mastitis after the more common forms of udder infection were brought under control. This would suggest the possibility that a too vigorous removal of the more common bacterial flora of the bovine mammary gland may lead to the opportunity for the bacteria of the soil and the environment to establish themselves in mammary glands and produce mastitis.

I would like to discuss with you some of my own experiences in this regard. The dairy herd in question had the best management among all the dairy herds we studied. The cows came into the barn only to be milked. Between milkings they were outside on cement. A portion of this area was bedded with straw and overhead shelter was provided.
In two years, from 1943 to 1945, *Streptococcus agalactiae* infection was reduced from 35% to 0%. This was accomplished before antibiotics were available so the elimination of the infection was accomplished by segregation of infected cows and eventual slaughter. Then, in 1945, a program to reduce staphylococcus mammary infection was started using segregation and intramammary therapy with antibiotics and chemotherapeutic agents. At the beginning of this phase of the work, about 50% of the cows were

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infected with pathogenic staphylococci (toxin producers and coagulase positive strains) although clinical mastitis was not a serious problem. The older cows with chronic staphylococcal infections did not respond well to treatment and, therefore, many of these cows remained infected until removed from the herd. However, by segregation of younger cows and early treatment of new infections, the incidence of staphylococcal mammary infections became reduced from 50% to 27% in a period of 4 years.

It was at this time that acute coliform mastitis made its appearance in the herd. During the first year, and before streptomycin was available to us, 10% of the herd experienced acute coliform mastitis. This meant 20 cases in one year. Three of these cows died and many others had to be destroyed. This severe coliform mastitis coming upon a very excellent mastitis control program was entirely unexpected and caused us to wonder if we had been responsible by distorting the more natural bacterial flora of mammary glands.

In 1959, we were able to begin experiments into the pathogenesis of coliform mastitis. The following is a brief summary of the most significant findings. In the original herd, several coliform types were involved, including both *Escherichia coli* and *Aerobacter aerogenes*. We choose the latter organism for our experimental trials for it is easily identified by simple culture media and therefore we could be more certain that is was the experimental strain when reisolated from milk of inoculated cows.

In the beginning we had to answer the question relative to number of germs and number of inoculations required to induce acute coliform mastitis. We know now that when a lactating gland is secreting leukocyte-free milk as few as 5 to 10 *A. aerogenes* germs can produce acute mastitis within 24 hours after their introduction via the "streak" canal. Normally, however, we employed larger doses to study the pathogenesis of the disease. For example one cow received 100 *A. aerogenes* into one quarter, 10,000 germs into another, and 1,000,000 germs into a third quarter. The fourth quarter was left as a control. From experiments of this type we demonstrated that the incubation period between introduction of the germs into glands secreting leukocyte-free milk and first clinical signs of mastitis is determined by dose-size. With less than 50 germs, it takes about 20 hours; with 1,000,000 germs, clinical signs of peracute mastitis develop 5 to 6 hours.

Heat-killed *A. aerogenes,* at a dose level of 1 million dead germs, injected into normal lactating glands stimulated a leukocytosis of several million cells per ml. of foremilk followed by a rapid return to normal. With the same number of live *A. aerogenes,* the leukocyte numbers reached 80 millions or more per ml. of foremilk. The glands became swollen and there were solid particles in the milk. During this period the milkweight (total milk) dropped about 50%.

However, if the inflammatory response was successful in completely destroying all *Aerobacter aerogenes* germs, the gland returned to normal within 7 to 14 days. On the other hand, if the initial inflammatory response was delayed for just a few hours, the cow became very sick and sometimes died. This was explained in that the coli-bacteria multiply rapidly in leukocyte-free milk and if there is a delay in the leukocyte response the bacterial population becomes very large. Finally, when the leukocytes do enter the gland and destroy the bacteria, a large quantity of endotoxin is released,

making the cow very sick. The endotoxin may damage the leukocyte-producing centres in the bone marrow and then a persistent leukopenia develops.
Another possibility in coliform mastitis, observed in our trials, was a too rapid fall in leukocyte numbers, after the initial inflammatory response and before all *Aerobacter aerogenes* were destroyed. When this happened, a second attack of acute mastitis developed. In some glands a chronic infection resulted and then acute mastitis appeared whenever the leukocyte numbers fell below 200,000 per millilitre of foremilk.

Thus, in our experience, coliform mastitis is a disease resulting from rapid multiplication of coli-bacteria in leukocyte-free milk. It appears, therefore, to be a disease of the normal lactating gland that has become accidentally invaded by coliform bacteria. The cow becomes sick from the endotoxin released when the leukocytes of the initial inflammatory response destroy the bacterial population.
In one experiment, after 500,000 *Aerobacter aerogenes* had been introduced into a normal lactating gland, we took 10 ml. of milk from the gland in 3 hours and again in 6 hours after the inoculation. The milk was centrifuged and smears were made of the sediment and stained by Wright's method (blood stain). Phagocytosis was demonstrated at the 3rd hour when bacteria were numerous but leukocytes were just beginning to enter the milk. By the 6th hour there were many leukocytes but the bacteria were too few for any evidence of phagocytosis to be seen. When the same numbers of *Aerobacter aerogenes* were introduced into lactating glands having leukocytes already in the milk from a pre-existing mild mastitis, the inoculated bacteria were prevented from rapid multiplication for the leukocytes began to destroy the bacteria immediately. Some increase in cell numbers could be demonstrated for next few milkings in such glands but the germs could not be reisolated and no signs of clinical mastitis developed. Mammary quarters producing milk with cell counts of 300,000 to 500,000 per ml. of foremilk were found to have a high degree of protection against experimental coliform mastitis. This level of cells in the milk would be representative of a mild inflammatory reaction and such milk would have a score of 10 seconds on the B.M.R. test for mastitis.

Now that there is great interest in mastitis control in many countries of the world, it is important to realize that coliform mastitis is a disease of the normal leukocyte-free mammary gland. Young cows have a good protection against coli-bacteria entering the mammary gland because the "streak" canal functions as an effective barrier. However, as a cow grows older and the "streak" canal of the teat becomes more patent, the accidental entrance of coliform bacteria into the mammary gland is more likely to take place. These older cows need the protection against coliform bacteria given by pre-existing leukocytes in the milk. Thus, if we insist in mastitis control that mammary glands of all cows must be free of all evidence of even mild inflammation, then it is possible and highly probable that the incidence of acute coliform mastitis will increase.
The cows used in our experimental trials did not develop any immunity to coliform mastitis. The cows were used many times in the experimental production of acute mastitis. The only protection against the disease appeared to be pre-existing leukocytes in the milk.

My story was not intended to disturb your considerations to improve milk quality by bringing mastitis under control. However, it was intended to serve as a warning and at the same time to encourage some tolerance for low level leukocyte counts especially in the milk of the older cows. Cell counts of 300,000 to 500,000 per ml. of foremilk will give a high degree of protection against the development of acute coliform mastitis in the event of accidental entrance of coli-bacteria into such mammary glands.

SAMENVATTING.

Op bedrijven waar het gelukt is mastitiden, waarbij streptokokken en stafylokokken een rol spelen, tot een minimum terug te brengen, kunnen plotseling ernstige vormen van coli-mastitiden bij de runderen optreden.
De sanering van deze bedrijven op eerstgenoemde mastitiden bij de runderen vond in de oorlogsjaren plaats zonder daarbij gebruik te maken van antibiotica. Door hygiënische maatregelen en zonodig door ruiming van moeilijk te genezen dieren werd *Streptococcus agalactiae* uitgeroeid. In 1945 werden ook de stafylokokken aangepakt door middel van isolatie en behandeling van de runderen met antibiotica en chemotherapeutica.
In het begin waren 50% van deze dieren stafylokokkendragers zonder dat deze alle aan klinische mastitis leden. Bij de oudere koeien was het succes gering, doch bij de jongere dieren daalde het percentage dragers in 4 jaren van 50% tot 27%.
Vanaf dat moment werd de coli-mastitis bij de runderen een probleem. Het eerste jaar leden 20 dieren hieraan (10%), waarvan er 3 stierven. In 1959 begonnen onze experimenten.
Wij vonden in de koppel *Escherichia coli-* en *Aerobacter aerogenes* mastitiden. We gebruikten alleen *Aerobacter aerogenes* bij onze experimenten en constateerden dat 5 à 10 bacteriën voldoende waren om in een lacterend kwartier, dat leucocyten-vrije melk produceert, binnen 24 uur coli-mastitis te doen ontstaan. Verder bleek ons, dat de snelheid van optreden van de ontstekingsverschijnselen afhankelijk was van het aantal ingebrachte bacteriën, mits de melk vrij was van leucocyten. Met 50 kiemen duurde het 20 uur en met een miljoen kiemen slechts 5 à 6 uur, voordat een klinisch waarneembare mastitis ontstond.
Een vermeerdering van het aantal leucocyten in de melk is zowel met levende als met dode bacteriën te bereiken. Door de aanwezigheid van een groot aantal cellen verdwenen de ingebrachte bacteriën soms snel, doch het duurde 7 à 14 dagen voor de veroorzaakte ontsteking genezen was. Vooral bij de dieren waar tijdens de besmetting geen ontstekingscellen in de melk voorkwamen, was de reactie van de uier t.o.v. de ingebrachte bacteriën vertraagd. Deze koeien werden daardoor erg ziek en stierven soms.
Wij nemen aan dat in leucocyten-vrije melk de bacteriën-vermeerdering snel verloopt en tot een grote populatie komt. In kwartieren waarvan de melk tijdens de besmetting 300.000 à 500.000 cellen per ml bevatte, had de besmetting met *Aerobacter aerogenes* veel minder succes. De aanwezige leucocyten elimineren blijkbaar de bacteriën. Naar onze mening is de uier van de jonge koe van nature door het enge tepel-kanaal voldoende beschermd tegen infecties die van buiten af komen. Bij oudere koeien zullen geheel of vrijwel geheel leucocyten-vrije kwartieren t.g.v. een besmetting grotere kansen op acute ontstekingen hebben. Zijn in de melk echter een matig aantal leucocyten aanwezig, dan verminderen deze risico's sterk.
Men zal bij een mastitisbestrijding dus moeten vermijden het aantal ontstekingscellen te ver terug te brengen.

Discussion

following the lectures of Prof. V a n d e r S c h a a f and Prof. Dr. O. W. S c h a l m.

Question: Dr. D. M. Z u y d a m (The Hague, The Netherlands):

I would like to put to Prof. V a n d e r S c h a a f two questions.
He mentions the interesting paper of S t u a r t and S l a v i n about P.P.L.O. infections in the udder of the cow. I am very much interested in this question, because mycoplasmosis plays an important part in the diseases of poultry. It is a base-infection of the respiratory tracts in birds and it causes the mycoplasmosis-gallinarum or chronic respiratory diseases.
For the campaign against mycoplasmosis in The Netherlands, an antigen of mycoplasmosis is made in order to detect the infected birds. The birds are tested by the wholeblood, or serum test. All flocks with a positive agglutination are eliminated. The first results in U.S.A. are very encouraging.
The two questions are:
First: do you think, Professor V a n d e r S c h a a f, mycoplasmosis or P.P.L.O. infection in the udder of the cow may be the base of secondary bacterial invasion?
The second point is: do you already consider making a mycoplasma- or P.P.L.O. antigen for milk or milkserumtest in order to find the mycoplasma infected herds?

Answer: Prof. V a n d e r S c h a a f (Utrecht, The Netherlands):

S t u a r t and S l a v i n of Weybridge have discovered that sometimes P.P.L.O. is a cause of mastitis. It goes from one cow to another; there are no bacteria. The difficulty for the diagnosis is that this micro-organism — this P.P.L.O. — is very difficult to cultivate. We have tried to cultivate it, but up till now we did not have any success; maybe in the future we will have more success.
About the second question: you ask a serological test which would be of help to detect the mycoplasmosis in the udders of the cows. I think it is time to go on to find the actual causes, which produce mastitis in the udders of cows. Maybe mycoplasmosis plays an important part, but up till now we do not know.
The question you ask me, Dr. Zuydam, I will pass to all members of this congress. S l a v i n and S t u a r t tried to cure the P.P.L.O. mastitis. They found penicillin was useless, because P.P.L.O. is resistant against penicillin. But also the tetracyclines had no result although the P.P.L.O. are sensitive for tetracyclines. The infection does not come back in the following lactiation period.

Question: Prof. D e d i é (Aulendorf, Germany):

Some remarks about the results of Professor V a n d e r S c h a a f.
In Aulendorf Professor K i e l w e i n and I used a tube-test. This test was more useful than the Schalm-test. We join your oponion about the alkaline reaction of the reagent. In Aulendorf we used a Schalm-test with a dedocyl-sulfate. This was buffered to pH 9, for chemical reasons. The action of dedocyl and sodium laurylsulfate is best at pH 9.
We did some first experimental work about the strain of *Miyagawanella bovis*, which we got from the Virus Institute of Tübbingen from Dr. S t r a u b. We used the complement fixation-test with the milkserum from mastitis cows in which we could not find bacteria in order to find *Miyagawanella*-infections. Up till now we are not sure we have found these infections in cows. This work is still in progress.

As to the results of Prof. S c h a l m, I should like to say we are controlling some herds very strictly for raw milk production. We are keeping the total amounts of leucocytes very low. I think below 100.000 cells pro m.l. We have few *Staphylococci*-infections, the *Staphylococci*-infections occur in about 10% of the cows. These herds are in very good state of control, but we never had any form of coliform mastitis in these herds. I think there must be other conditions, that will cause the coliform mastitis.

I should like to put Prof. S c h a l m two questions:

1. Do you know in which time of lactation this colimastitis occurs?
2. Which disinfectant was used in these herds?

Answer: Prof. S c h a l m (Davis, U.S.A.):

The coliform mastitis we speak about is not common and only occurs in these herds, in which the mastitis control is done for several years. The cows have had in average about four lactation periods. The productive cows stay longer in the herds.

We have the opinion, that especially in the older cows the coliform mastitis occurs. I think the coli enters the udders by way of the teats. We know nothing or little about the coli coming in the udder by way of the bloodstream. The coliform mastitis most occurs in a short time after refreshing, but that is not necessary.

As for the disinfection the teatcup of the machine was dipped between every milking into a chlorine solution, also the teats were tipped in chlorine. The sanitation was excellent.

We were dealing with an unusual situation. If all experiences are correct, coli grows only in leucocyte- free milk. A leucocyte count of 300.000 to 500.000 leucocytes per milliliter, which is subnormal, is highly protective against colimastitis.

Remark: Dr. K l a s t r u p (Ringsted, Denmark):

Most cases of coliform mastitis occur some days after refreshing. As far as I know we have the greatest amount of cells in the milk, some days after refreshing and I therefore can not understand that in this period most cases of coliform mastitis occur.

Answer: Prof. S c h a l m (U.S.A.):

The normal cells in the colostrum of the cows are no leucocytes. When I speak about leucocytes, I mean the polynuclear leucocytes. Colostrum is rather low in the number of leucocytes. Maybe this is the solution of the problem.

Control of Mastitis by Hygiene.

by F. K. NEAVE, F. H. DODD and R. G. KINGWILL*)

National Institute for Research in Dairying, Shinfield, Reading.

Introduction.

Dr. Brus: *Mr. Kingwill, I was very glad to hear that you will address the Conference on the Control of mastitis by hygiene.*
From my co-worker Dr. Jaartsveld, I have already heard about your work and I am very happy to welcome you here.

Introduction

From the results of our work at Shinfield and other published data we believe that there are good prospects of developing hygiene into an effective means of controlling udder infection by the common pathogens. This is not a new idea, but there is no proof that any hygiene system so far developed is effective.

The value of hygiene systems is based on the hypothesis that infection by the common pathogens is normally caused by organisms transmitted during milking on hands, udder cloth and teat cup liners, and that this transfer can be largely prevented. Research in our institute herd showed that, using cows with healthy teat skin, this transfer could, to a very large degree, be prevented. To find out whether this result could be achieved under a range of commercial conditions and to measure its effect on new infection, a field trial was planned.

Aim.

The aim of the experiment is to compare the **incidence of new udder infection** when no special hygiene is practised with the incidence when using the highest practical level of hygiene at milking time.

Methods.

The spread of bacteria by milking is very greatly reduced by the use of a chemical disinfectant for hand and udder disinfection and the pasteurisation of teat cup liners. A solution of chlorhexidine digluconate (Hibitane; 100 p.p.m.) is used to disinfect the milker's hands between each cow and for washing each cow's udder with either an individual paper towel or sterile cloth before milking. A pasteuriser (see below) circulates approximately 2 litres of water at 85° — 90° C through the teat cup liners and milk tube for 5-8 seconds before each cow. Immediately after milking the teats of each cow are dipped in a solution of Hibitane (5000 p.p.m.). These methods are applied to each cow in the hygiene herds by the following milking routine throughout the trial year.

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1. First examine foremilk using strip cup;
2. dip both hands in disinfectant solution (Hibitane; 100 p.p.m.);
3. wash udder using paper towel or sterile cloth and disinfectant solution (Hibitane; 100 p.p.m.);
4. milk cow with automatically pasteurised teat cup. (proceed with work on other cows until milking complete);
5. dip both hands in disinfectant solution (Hibitane; 100 p.p.m.) before machine stripping and removing teat cups;
6. remove teat cups and place in pasteuriser;
7. dip each teat in disinfectant solution (Hibitane; 5000 p.p.m.).

The routine applied in the control herds is as follows:

1. First wash udder with cloth and plain warm water;
2. examine foremilk using strip cup;
3. milk cow;
4. no attempt to rinse or disinfect teat cups or hands between cows, except to remove gross soiling.

Diagnosis of infection.

Diagnosis of infection is based on the recovery of bacteria from milk samples, physical appearance, Whiteside test reactions, and cell counts when other tests are inconclusive. Two individual quarter milk samples from each cow were examined at the start of the experiment or at calving, and quarter samples from each cow are examined at four-monthly intervals or at the end of lactation.

Confirmatory milk samples are examined when a change of infection is indicated and repeated examination of samples made until the position is defined. Strains of *Staph. aureus* are phage typed to aid the diagnosis of new infection. Before therapy two milk samples are taken by farm staff, and Institute staff collect post-treatment samples approximately 21 and 28 days after therapy to find out if the infection has been eliminated.

The efficiency of hygiene.

To measure the effectiveness of the hygiene routine or to indicate the reasons for failure to prevent the spread of bacteria, swabs of each cow's teats are examined at the start of the experiment and at four-monthly intervals. At weekly intervals in each hygiene herd teat swabs have been examined from a few random non-infected cows and swabs from teat sores and milker's hands and teat cup liners after pasteurisation.

In all herds teats were examined at the start of the experiment and at 4-monthly intervals of more frequently, and a record made of teat lesions and orifice erosion.

General management factors.

No attempt was made to alter the general management or milking technique in any herd, but details of management and changes were recorded. At the start all milking machines were checked and corrected, if necessary, to provide 15" Hg vacuum, effective pulsation and a controlled reserve of vacuum.

Clinical mastitis and therapy.

Antibiotic therapy is restricted to clinical cases of mastitis, and farmers do not have details of sub-clinical infections. Veterinary surgeons examine any severe cases of mastitis, but with the collaboration of all practitioners a standard antibiotic therapy is available for cases where only clots in the milk or mild induration is detected. In all cases the milker takes two milk samples before inspection, and gives 1 tube of antibiotic per affected quarter, repeated twice at 48-hour intervals.

Farm visits.

Field staff visit each herd weekly to check the milking routine, collect data and samples, and service the pasteurisers. Senior staff visit herds for all teat examinations, to investigate problems and for general supervision. Faults in equipment are corrected before the next milking.

Recording of data.

A specially designed punched card is used to record every item of information for each cow, and this provides a means of deciding when confirmatory samples or swabs are required. Further cards are used to record and analyse each infection and each course of therapy.
A weekly sampling programme is entered in each herd diary, and field staff take the diary for each visit and record in it all observations.
Milkers are provided with a clinical record sheet to record mastitis and antibiotic treatments.

Design of experiment.

In order to guarantee the pasteurisation of teat cups in the absence of supervision it was decided to make the pasteurisation equipment automatic, fitting it as an integral part of the milking equipment (see below).
This decision was useful in that it meant that the experiment was testing a practical process but it limited the experiment to parlour milked herds. It was further decided that these milking parlours should be of the one unit per stall type because of the long unit-idle time. These considerations together with the difficulties of measuring residual effects with crossover design led us to choose a design with the treatment comparison made between herds.
Available data indicated that 9 herds per treatment were required for a year to give an 80% chance of measuring a 40% reduction in the incidence of new infection at the 5% level of significance. To limit costs and allow for withdrawals the experiment is to be run over two years, commencing with 7 herds on each routine. Herds were selected according to the number of cows, type and level of infection and cooperation of personnel. Before the allocation of treatments all farmers agreed to accept either control or hygiene routine. The only reasonably blocking criteria was the presence or absence of *Streptococcus agalactiae* infection, and the experiment is a completely random design with random allocation of treatment to herds in these two groups.

The pasteuriser.

The pasteuriser consists of an electrically heated water bath containing a basket to hold the teat cups, and a releaser. The position of the lid of the bath governs the cycle of operation. When the lid is fully closed milking vacuum is applied to the teat cups. Raising the lid seals off the milking vacuum and physically isolates the long milk tube from the connection to the milk line. The teat cups may now be placed in the basket with their mouths in hot water.

When the lid is lowered to a pre-determined mid-position vacuum is applied via the releaser to the teat cups, and water is drawn through the teat cups, clawpiece and milk tube.

When approximately 2 litres of water has entered the releaser a float rises to displace a ball valve. Vacuum enters a diaphragm motor which releases a spring catch, allowing the basket to rise. The teat cups are lifted clear of the water, and can drain, and at the same time the vacuum supply to the releaser is blanked off.

When the milker is ready, raising the lid to remove the cluster and fully closing the lid resets the vacuum for milking and positions the basket for the next cycle.

Discussion.

From Table V, which gives provisional results of new udder infections in the first eight months of the trial, it is clear that there are uncontrolled factors affecting the new infection rate within the two groups of herds that have a greater influence than the experimental treatment. Nevertheless, the total of new infections in the hygiene herds appears to have been reduced by 50% compared with the control herds, and when cross-infections are eliminated the reduction is 60%. This result is encouraging, but it is obvious that in spite of hygiene, new infections occur frequently in herds 1, 2 and 4.

Measurements of the pathogens on teat cup liners after pasteurisation, and the outside of teat cups (indicating the level of hand contamination) show that the hygiene routine is satisfactory in these respects. (Table III). The more important criterion is the presence of staphylococci on the teats of non-infected cows, and although Table II shows that these were reduced by the routine the results have not been as good as were obtained in the research herd.

The presence of staphylococci on teats must be partly due to the high incidence of chapped teats in the hygiene herds (table IV), a result that was not anticipated. Although it would appear impossible to have an effective hygiene routine unless the skin of the cow's teats is healthy, the presence of chaps in both hygiene and control herds is not closely related to the incidence of new infection (see tables IV en V).

Our examination of the results has failed so far to reveal any major factor influencing the new infection rate within the treatment groups and it is quite possible that this is due to the relative infectivity of the different strains of pathogens in the herds.

Table I.

Description of 14 herds used in a field experiment.

Herd	No. of cows	Breed	Average Age (lact.)	Proportion of cows (%) infected with				All types	% cows with chapped teats	Milking Machine		Housing in winter
				Staphylo-cocci	Str. agalactiae	Str. dysgalactiae	Str. uberis			Make	Type	
1	30	Friesian	3.0	29	29	7	21	59	57	G	In-can	covered yard
2	46	Friesian	3.3	59	0	6	6	63	12	A-L	Jar-can	semi-covered yard
3	42	Guernsey	3.4	40	12	7	12	52	86	G	In-can	covered yard
4	42	Ayrshire	3.0	66	0	17	6	69	26	S	Pipeline	semi-covered yard
5	40	Friesian	2.6	55	0	8	4	60	33	A-L	In-can	semi-covered yard
6	51	Friesian	3.4	56	0	16	2	66	37	V	In-can	out-wintered
7	39	Shorthorn	3.7	59	38	10	0	74	54	A-L	In-can	out-wintered
8	32	Ayrshire	3.6	19	28	3	16	50	13	G	In-can	covered yard
9	34	Ayrshire	3.0	47	0	0	9	50	79	S	In-can	semi-covered yard
10	40	Friesian	3.0	58	28	3	3	68	59	A-L	In-can	semi-covered yard
11	26	Jersey	3.0	73	0	15	35	85	17	A-L	In-can	semi-covered yard
12	32	Friesian	2.3	84	0	3	3	84	19	A-L	Pipeline	covered yard
13	57	Friesian	3.7	32	0	9	44	60	11	A-L	In-can	semi-covered yard
14	45	Friesian	2.9	33	0	6	4	36	22	W	In-can	out-wintered

Table II.

Percentages of swabs with Staph. aureus from teats after milking. (plated direct)

	Hygiene herds	Start of Expt.	After 4 months
No.	1	96	14
	2	95	41
	3	100	12
	4	100	40
	5	100	4
	6	100	60
	7	100	—
		Mean % 99	28

Table III.

No. of swabs with Staph. aureus from teat cup clusters after pasteurisation.

Hygiene herds No.	Inside teat cup liner No. swabs	No. +ve	Outside of teat cups No. swabs	No. +ve
1	17	0	17	1
2	22	2	23	6
3	24	0	24	0
4	28	0	27	0
5	22	1	22	6
6	18	0	18	1
Total	131	3	131	14

Table IV.

Percentage of cows with healthy teat skin, chapped teats, and „infectious" teat sores.

Herd No.	Breed	% Healthy Teats Date of examination			% Chapped teats Date of examination			% „Infectious sores" Date of examination		
		June/July '62	Oct./Nov. '62	Feb. '63	June/July '62	Oct./Nov. '62	Feb. '63	June/July '62	Oct./Nov. '62	Feb. '63
	Hygiene herds									
1	Fr	13	47	15	57	34	70	43	3	26
2	Fr	61	46	4	12	41	89	4	0	27
3	G	0	14	3	86	68	94	10	0	0
4	Ayr	33	49	21	26	40	68	17	7	3
5	Fr	40	29	21	33	32	58	13	4	21
6	Fr	49	42	3	37	50	98	4	0	5
7	Sh	5	—	—	54	—	—	69	—	—
Mean %		33	38	11	43	44	79	23	2	14
	Control herds									
8	Ayr	48	47	59	13	13	0	0	3	24
9	Ayr	3	17	3	79	43	71	18	13	42
10	Fr	10	14	17	59	44	78	27	19	8
11	J	4	57	29	17	24	46	0	0	0
12	Fr	25	47	35	19	40	58	9	5	16
13	Fr	67	0	0	11	9	88	0	100	47
14	Sh	40	11	0	22	51	83	5	27	72
Mean %		34	28	20	31	32	61	8	24	30

Table V.

New infections occurring in the 12 months of the field experiment. (Provisional Data)

			Total new infections						New infections excluding possible cross infections within udders					
		No. cows in milk during whole or part of period	Staph. aureus	Strep. agal.	Strep. dys.	Strep. uberis	Others	Total	Staph. aureus	Strep. agal.	Strep. dys.	Strep. uberis	Others	Total
Hygiene herds	1	42	26	3	4	11	2	46	16	2	1	8	2	29
	2	58	55	0	24	9	7	95	16	0	21	3	7	47
	3	55	8	0	0	0	0	8	7	0	0	0	0	7
	4	62	43	0	4	4	11	62	18	0	3	1	7	29
	5	51	19	0	4	3	1	27	8	0	1	2	1	12
	6	59	18	0	4	7	0	29	8	0	2	3	0	13
	Total	327	169	3	40	34	21	267	73	2	28	17	17	137
Control herds	8	43	19	2	16	18	0	55	13	1	11	11	0	36
	9	42	43	0	11	4	1	59	29	0	10	3	1	43
	10	51	9	10	26	9	0	54	7	5	24	6	0	42
	11	32	26	0	3	11	1	41	5	0	1	7	1	14
	12	52	79	0	54	3	2	138	39	0	38	3	2	82
	13	58	14	1	29	25	7	76	11	1	16	10	6	44
	14	65	41	0	35	9	9	94	27	0	23	7	6	63
	Total	343	231	13	174	79	20	517	131	7	123	47	16	324

SAMENVATTING.

De spreker, Mr. R. G. K i n g w i l l, houdt zich in deze voordracht vooral bezig met de invloed van hygiënische maatregelen van de melker, de uier en de machine op het voorkómen en ontstaan van uierontstekingen bij runderen.
De desinfectie van handen en uier geschiedt met chloorhexidine digluconaat (100 d.p.m.); det tepels worden direkt na het melken gedompeld in chloorhexidine (5000 d.p.m.) en de tepelvoeringen worden gedurende 5—8 seconden in stromend water van 85° C verwarmd.
De waarde van hygiënische maatregelen berust op de hypothese dat de infectie — veroorzaakt door de uier-pathogene microörganismen — gedurende het melken ontstaat via de handen, uierdoek en tepelvoeringen. Met behulp van hygiënische maatregelen kan men de overdracht van microörganismen grotendeels beperken, vooral indien de huid van de runderen in een goede conditie is.
Het doel van dit onderzoek is het vergelijken van het ontstaan van het aantal nieuwe gevallen van uierontsteking op bedrijven waar onder extra goede hygiënische omstandigheden wordt gemolken en op bedrijven waar deze maatregelen niet worden genomen, gedurende een tijdvak van een jaar.
Een pasteuriseerapparaat werd geconstrueerd waarmee het mogelijk is ongeveer 2 liter water van 85—90° C gedurende 5—8 seconden te doen circuleren door de tepelvoeringen en de melkslang. Deze desinfectie wordt gedurende het melken en na afloop ervan voor elke te melken koe toegepast.
De hygiënische maatregelen, welke op de z.g. hygiënische bedrijven werden toegepast, waren de volgende:

1. Het onderzoek van de eerste melkstralen met behulp van een voormelkbeker;
2. beide handen van de melker worden gedompeld in een hibitane oplossing (100 d.p.m.);
3. de uier wordt met hibitane (100 d.p.m.) door middel van een steriele doek (papier) gewassen en gedroogd;
4. de koeien worden gemolken met gepasteuriseerde tepelbekers;
5. voordat het apparaat afgenomen wordt, worden beide handen in een hibitane oplossing (100 d.p.m.) gedompeld;
6. de tepelbekers worden in het pasteurisatieapparaat geplaatst dat half automatisch werkt;
7. de tepels worden gebracht in een hibitane oplossing (5000 d.p.m.).

Op de controlebedrijven werden de volgende maatregelen getroffen:

1. De uier wordt met warm water gewassen en met behulp van een doek afgedroogd;
2. de eerste stralen worden met behulp van een voormelkbeker onderzocht;
3. de koe wordt normaal gemolken, zonder enige bijzondere hygiënische maatregelen.

De diagnose van uierontsteking wordt gesteld aan de hand van het bacteriologisch melkonderzoek, het uitwendige aspect van de melk en de Whiteside-reactie, eventueel ook door celtelling.
Om het effect van de hygiënische maatregelen te controleren werden met behulp van steriele „swabs" de tepels van elk rund bacteriologisch onderzocht, evenals de handen van de melkers en de tepelvoeringen. Dit onderzoek gebeurde elk kwartaal. Alleen de gevallen van klinische mastitis werden met antibiotica behandeld.
Met behulp van een ponskaartensysteem werden de gegevens genoteerd.
Tabel I geeft een overzicht van de 14 bedrijven die deelnamen aan de praktijkproef.
Tabel II geeft aan welk percentage „swabs" van de tepels der runderen op de hygiënische bedrijven, 4 maanden na het begin van het experiment, *Staphylococcus aureus* bevat vergeleken met de „swabs", genomen bij het begin van het experiment.
Tabel III geeft aan het aantal swabs met *Staph. aureus* die genomen zijn na de pasteurisatie van de tepelvoeringen uit het inwendige en uit het uitwendige van de tepelvoeringen. Hieruit blijkt dat de isolatie van *Staph. aureus* veel frequenter

plaats vindt aan de buitenkant van de tepelvoering dan aan de binnenkant van de tepelvoering. Tabel IV toont dat het aantal runderen met kloven op de spenen en met tepellaesies op de hygiënische bedrijven aanmerkelijk hoger in aantal zijn dan op de controle-bedrijven.
Blijkbaar heeft de desinfectie van de tepel een nadelige invloed op de gaafheid van de tepelhuid.
De laatste tabel, een voorlopige mededeling, geeft aan dat het aantal positieve bacteriologische onderzoekingen op de z.g. hygiënische bedrijven aanmerkelijk lager is dan die op de controle-bedrijven Dit geldt speciaal t.o.v. de isolatie van het aantal stafylokokken, *Str. agalactiae, Str. dysgalactiae* en *Str. uberis.*

SUMMARY.

A preliminary report of a controlled field experiment with 700 cows in 14 herds to measure the effect on the incidence of new udder infection of a hygienic milking routine. The routine includes disinfection of hands and udders with chlorhexidine digluconate solution (100 p.p.m.), pasteurising teat cup liners between each cow (85° C. for 5—8 seconds) and teat dipping in chlorhexidine digluconate (5000 p.p.m.) immediately after milking.
The results of examination of swabs from teats, and teat cup liners are discussed in relation to new udder infections found by examination of milk samples for bacteria, Whiteside test reaction and cell count.

Discussion,

following the lecture of Mr. K i n g w i l l.

Question: Prof. D e d i é (Germany):

Mr. K i n g w i l l, may I put you two questions?

1. If you use heat to disinfect the teat-cups without use of rinsing water, would you not get milkstone in your cup-liners?
2. When you use a disinfectant after milking without drying the teats, don't you get some irritation at the teats of the cows?

Answer: Mr. K i n g w i l l (Reading, England):

We have not found that milkstone formation has occurred. I cannot explain this, perhaps it is, because there is always rinsing between two cows. The liners of the milking-machine have lasted only about half their normal length of life. Further, I think that synthetic rubber will be better for this than natural rubber but we have done little work yet. It is not necessary to cool these cups. We have seen no harm of the cow and no irritation.
As to your question of wet teats. We have not found a direct relation between the wet teats and irritation.

Question: Prof. V a n d e r S c h a a f (The Netherlands):

Mr. K i n g w i l l, I should like to ask you about the temperature-coefficiënt of the disinfectant. There is a great difference between the different disinfectants. Fenol acid has a low temperature-coëfficiënt and chlorine has a very high one. Also the chlorhexidine has a high temperature-coëfficiënt.
When I saw the dipping of the hands of the people we have seen in your film, I asked myself, what is the temperature-coëfficiënt of the disinfectant? Is it perhaps five degrees, ten degrees, twenty-five or forty-seven

degrees? For the disinfection of the hands, it was best the temperature of the disinfectant is thirty-seven degrees.
Also I'm interested in the temperature of disinfectants for disinfection of the teatcups. According to the experience of Dr. J a a r t s v e l d and me, in which we used 0,4% chloramine at about 20 degrees, we got a total disinfection of germs of the skin of the hand within three minutes. You didn't get a whole sterilization of the cloths and I ask how is that possible?
Maybe you have a lower temperature of the disinfectant?

Answer: Mr. K i n g w i l l (England):

About your first question. The disinfectant for the first cow was prepared at a high temperature, about 40 to 45 degrees C°. The temperature declined, perhaps at the end of milking to 20 degrees. When we made the comparison for hand disinfection the temperature of the disinfectant was about 40 degrees C°.
For disinfection of the teat-cups a fresh solution of disinfectant was always used. In this way the disinfectant was not contaminated except from the milk of the clusters.

Question: Dr. E d w a r d s (Compton, England):

We have seen this disinfection-machine in a milking-parlour, but I should like to ask you, if it's possible to use this machine in a milking cow shed. The machine would have to be moved over some distance.
In these results we have seen, it is perhaps a disappointment to many of us who have worked in mastitis control to find, that the staphylococci infection is the most difficult infection to control by disinfection.

Answer: Mr. K i n g w i l l (England):

It's possible to get a moving disinfection-machine. In a researchherd we started this work in the cow shed, and developed a machine on wheels. The hot water is heated on butagas.
About the second question; I agree with you about the disappointment in not reducing the staphylococci infection with these methods of disinfection. One point is that the disinfection of teats is always incomplete. This is one important thing. You will be familiar too with the paper of D a v i d s o n. I think from his work is does seem likely that if one can reduce the level of udder infections, the risk of survival of staphylococci will be less. We will hope that as we make a little more progress with the disinfection of the teats, we will get better results.
There is another aspect too. We have made no attempt to segregate or reduce the chronic infected cows nor to use antibiotics in the infected cows. Antibiotic-application was restricted to clinical cases of mastitis. In common herds clearly one should use all the possibilities for controlling mastitis. One would be wise, one would eliminate the cows with chronic mastitis and one would disinfect as well as possible.

Question: Dr. K y r k j e b ö (Oslo, Norway):

Do you find that hibitane protects the teats against teats lesions better than plain water, and what about the skin-contact between the different disinfectants?

Answer: Mr. K i n g w i l l (England):

On the first question the answer is „no"; we have no evidence that hibitane gives a better protection of the teats. In a survey we have seen that by hibitane we got more teat lesions than by hypochlorite. Maybe this was caused by the high concentration of a detergent that was added

to hibitane. Now the hibitane is diluted in less detergent and I hope in the future we get better results.
The second question was about the skin contact between the different disinfectants. Chlorine appeared to be slightly inferior to hibitane. Possibly, but I have no evidence, because there is an accumulation of the bactericide chlorhexidine on the skin. This is one possibility.

Remark: Prof. V a n d e r S c h a a f (The Netherlands):

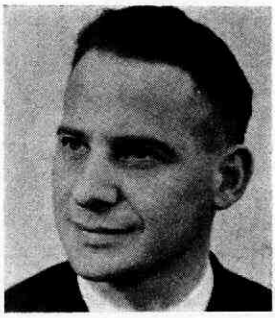
I should like to consider further the matter of disinfection after milking. There is a very good work of Dr. T h i p a t h y of the Cornell University, a thesis of the former year. Systematically disinfectants — also G-11 — were tested in disinfection the teats after milking.
The result was, that disinfection after milking had no results at all on the number of bacteria, found at the moment for the following milktime. So the contamination by the bacteria and other agents is so heavy that disinfection after milking has no worth.
But there is a second thing and that is that disinfection after milking gives more cracks and lesions of the teats and that should be avoided. Keep the skin of the teats in good health. That is the first thing we have to do and therefore we should avoid every disinfection.

Answer: Mr. K i n g w i l l (England):

May I answer the second point.
I agree that the health of the skin is very important and we have been aware of the danger of disinfecting the teats after milking. We have to do more work, but we have found that the aqua solution of hibitane is not harmful.
Concerning the report you have spoken of, I must say, we have found the reverse. We have made comparisons of disinfectants for washing the udder and teats of the cow, and we have dipped teats after milking. We have taken swabs of the udder and teats, firstly before the cow is milked and after the cow has been washed.
It does appear that teat dipping has a distinctly beneficial effect, but maybe it is better we will discuss this problem later on.

The development and the application of the Brabant Mastitis Reaction (B.M.R.).

by F. H. J. JAARTSVELD*)



In The Netherlands every province has its own Animal Health Service. There are eleven Provincial Animal Health Services; they are real farmers' organisations.
After the second world war the activities started with the eradication of tuberculosis, soon followed by the control of brucellosis. In the meantime the Animal Health Service got the supervision of the artificial insemination of cattle and swine, and began an organization to eradicate swine- and poultry diseases. Veterinary surgeons and farmers can be assisted in individual disease problems.
For the control of brucellosis of cattle, blood and milk-samples are examined on brucellosis antibodies. At least every quarter of a year milk-samples are taken at the dairy-factories out of all cans for the examination on brucellosis-antibodies, with the Abortus Bang Ring-reaction (A.B.R.). In order to transport these milk-samples easily, we make use of milk-chests, in which several tube-containers are placed, each containing 100 tubes.
By means of an instrument milk-samples are taken out of every can delivered at the dairy-factory. A milk-sample of 1 ml is transferred to a tube. When the tube-containers arrive at the laboratory one drop A.B.R.-antigen is added to each of the 100 tubes with a special drop-apparatus. The antigen and the milk are mixed, placed at 37° C. for one hour, after which the result of the A.B.R. of the milk-samples is noted.
It is also possible to use the same milk-samples for the mastitis-reaction. The appearance of a great number of cells in the milk is the most important criterion for mastitis. Therefore the milk is mixed with T-pol or sodium laurylsulfate. Those reagents are capable to break open the nuclei of the cells (leucocytes). The desoxyribosenucleic acid (DNA), originating from the nuclei of the cells in the milk, causes a sharp rise in viscosity and the more cells the milk contains, the more ropy the mixture is. The ropiness is measured by means of the Brabant Mastitis Reaction in determining the rate of flow through capillary tubes.
0.6 ml of milk and 0.4 ml of T-pol 414 or sodium laurylsulfate 2% are mixed and put in the funnel. There is a relation between the time of flowing through the capillaries and the number of cells in the milk. The time of flowing through is measured after 5, 10, 20 and 60 seconds. The samples still remaining in the funnels after 5 seconds are marked with one dot (•), the samples still in the funnels after 10 seconds are marked with a second dot (••). Those that are still in the funnels after 20 seconds get

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three dots (•••) and those that do not flow out after 60 seconds get four dots (••••).

In order to apply the B.M.R. in a uniform way the milk-samples with which the A.B.R. is done, are shaken very well afterwards. In this way the cream is mixed with the milk and the leucocytes are homogeneously mixed in the whole milk-sample.

By means of an sucking-apparatus, the total volume of the milk-samples in the tubes is reduced to 0.6 ml milk, so every tube contains 0.6 ml milk. Afterwards with a pipetting-apparatus, 0,4 ml of sodium laurylsulfate 2% is added to the 0.6 ml of milk.

The liquids are mixed and decanted by means of a perforated interplate in 100 flow-through capillaries, which are fixed on a plate covered with a sheet of foam-rubber. In this way the flow of the capillaries is blocked. In order to register the B.M.R. the apparatus with 100 flow-through capillaris is lifted up and placed below a film camera and after 5, 10, 20 and 60 seconds a picture is taken.

The milk-samples which flow through the capillaries within 5 seconds, have a negative B.M.R. The milk-samples which do not flow through the capillaries after 5 seconds get one dot, after 10 seconds two dots and so on.

There exists a very close relation between the B.M.R. and the mean total of cells in the milk-samples. The B.M.R. is called positive if 3 or 4 dots are recorded, doubtful if 1 or 2 dots are recorded and negative if no dots are recorded. This reaction is done with milk-samples out of cans, pails or quarters of the udder. Six laboratory workers are capable of examining 10.000 milk-samples in one hour by means of the B.M.R. in a simple, accurate and cheap way.

A regular examination by means of the B.M.R. of all cans delivered at the dairy-factors gives a clear impression of the farms with serious or no complaints of mastitis. The B.M.R. is a very simple reaction, therefore the different laboratories may gain the same results with the same milk-samples. By means of the B.M.R. of the milk out of the cans delivered at the dairy-factories it is possible to indicate the farms with mastitis problems.

By means of the B.M.R. of the pail- or quartermilk-samples one is capable to indicate the cows with mastitis at these farms. The primary cause of mastitis in most cases is a traumatic one, for example hurts and contusions. Bacteria play a secondary role. If it is possible to find and to take away these causes the number of cases of mastitis will sharply decrease.

The B.M.R. is suited also to the determination of the leucocytes of blood-samples. At first the blood-samples with heparin were diluted 1:25 with water and then the B.M.R. was carried out. This method gave bad results, especially if the blood-samples were diluted some time before the B.M.R. was performed.

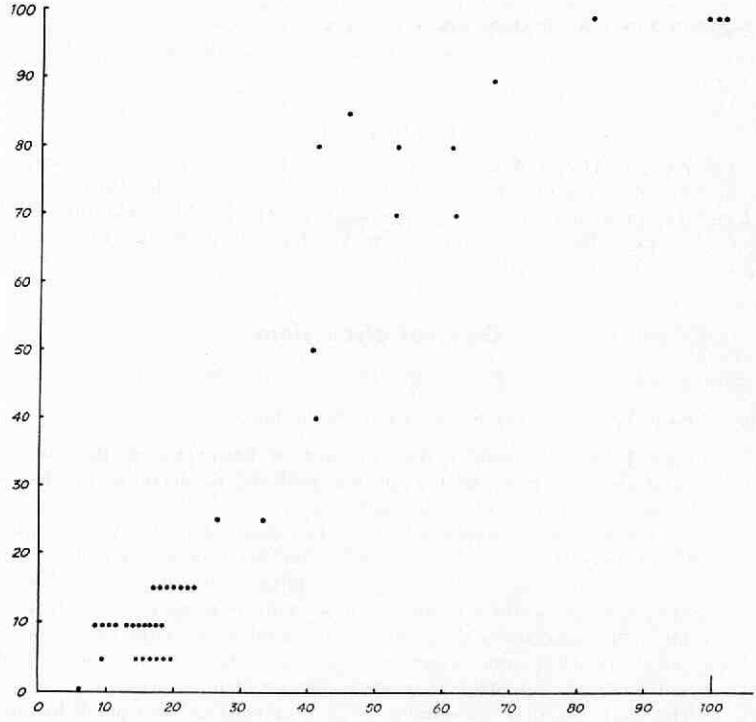
We got better results if the blood-samples were diluted 1:20 with saline (0,9%), before the B.M.R. was performed.

The graph shows the relationship between the B.M.R. on the diluted blood-samples and the total leucocyte-count per mm^3.

In addition to the examination for brucellosis and mastitis it is also possible to examine the milk-samples for the presence of antibiotics. Therefore rectangular plates are made with the same surface as the tube-containers. In the future we will use plastic rectangular plates for this purpose,

Relationship of the B.M.R. on diluted blood samples and the total leucocyte-count.

10 ml. of blood mixed with one drop heparine 5%, afterwards diluted 1:20 with saline-solution (0,9%).



*) x 1000 leucocytes per mm^3.

which are suited also to the bacteriological examination of milk-samples. Approximately 160 ml Agar medium is mixed with 25 ml *Sarcina lutea* culture and poured out in this plate. After that a "punching-apparatus" with 100 legs is placed on the bottom of the plate.
When the agar has cooled off, the punching-apparatus is removed from the plate and 100 little holes stay behind.
With an "Antibiotic-drop-apparatus" out of every tube of the milk-chest 2 of 3 drops of milk are removed and put in the corresponding hole of the agar. The plate is placed at 37° C for about 20 hours.
All milk-samples with 0.02 E penicillin or more per ml. have a clear zone of inhibition.

SAMENVATTING.

De uitvoering van de B.M.R. wordt beschreven zoals deze reeds eerder werd gepubliceerd.
Tevens wordt er op gewezen dat de B.M.R. in principe te gebruiken is voor het onderzoek op het aantal kernhoudende cellen in het bloed. Hoewel deze methode niet de nauwkeurigheid van de bestaande methoden heeft om het aantal leucocyten in het bloed te tellen (telkamer van Bürker), geeft ze een indruk van het totaal aanwezigee kernhoudende cellen.

Het te onderzoeken bloed wordt met behulp van heparine onstolbaar gemaakt. Daarna wordt het met fysiologische NaCl-oplossing verdund tot b.v. 1 : 20, waarna met deze oplossing de B.M.R. wordt uitgevoerd (zie grafiek).
De melkmonsters die onderzocht worden met behulp van A.B.R. kunnen door een eenvoudige techniek eerst op het voorkomen van groeiremmende stoffen worden onderzocht, vóórdat de B.M.R. wordt uitgevoerd.
Voor de uitvoering van het onderzoek kan verwezen worden naar de laboratoriumdemonstratie, blz. 95 e.v.

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General discussions

on September 25th, 1963; at the end of the first day of the Congress.

Remark: Prof. V a n d e r S c h a a f (The Netherlands):

First I like to consider the presence of leucocytes in the milk. It is dangerous, as Prof. S c h a l m has told us, to decrease the leucocytes below a minimal level in the milk.
I have had some experience about leucocytes, but this was a quite different problem. It was the problem of bacteria in vaccinia. Formerly vaccinia contained some millions of staphylococci pro gramme of vaccinia. After the first world war the vaccinia could be made better as it was told. Therefore vaccinia with a very small number of staphylococci was produced. In 1927 some cases of encephalitis have been seen in Holland and I had the opportunity to study this problem.
The post vaccinal encephalitis was supposed to be caused by another virus. It was also possible that the virus had become more virulent than before, but the real cause was the very low content of staphylococci in the vaccine. The strain of vaccinia, which was very mild and which had never given an encephalitis contained many staphylococci.
I tried to make the vaccinia more mild by mixing it with staphylococci. The staphylococci give a zône of leucocytes round the place of injection. This is the reason why the old vaccinia virus was much milder, than the new vaccinia virus which had a very low content of staphylococci. The same results I got when I injected vaccinia with a few staphylococci mixed with turpentine. In this way it was possible to render the neurolapine milder.
The leucocytes prevent the generalization of the virus and this is the same problem Prof. S c h a l m has found with coliform mastitis.
For the mastitis campaign it is important to know how far one can reduce the total number of leucocytes in milk without permitting every cow to get mastitis of bacterial origin.

Question: Miss W e i g t h (Hanover, Germany):

We have also some experience with the coliform mastitis and we found also that the total bacteria used for an infection is very important to get an inflammation of the udder. We had to use more bacteria to get an infection than Prof. S c h a l m. All test objects were cows of the slaughterhouse. Most of them had a severe or chronical mastitis and therefore they

had many leucocytes in the milk. That was the reason that we had to take more bacteria to get an inflammation of the udder. We used about 20.000 micro-organisms.

I should like to ask Dr. S c h a l m which kind of mastitis you found in U.S.A. We found acute cases of mastitis and more chronical cases of mastitis, especially in case of wounds on the teats. Is it possible that the *Aerobacter aerogenes* also causes a chronic form of mastitis?

Answer: Prof. S c h a l m (U.S.A.):

We did not find chronic mastitis caused by *E.coli,* but with *Aerobacter aerogenes* — even in our experimental cows — we had one animal that failed to recover completely from *Aerobacter aerogenes*-infection. This cow has been infected several times. She carried the infection for months. We had to incubate the milk to prove that the *Aerobacter aerogenes* was still in the udder.

It must be possible that if a cow is infected with *Aerobacter aerogenes* she fails to recover completely and carries this infection for months. We must incubate the milk to prove that the *Aerobacter aerogenes* is still there, but periodically about every three weeks we get a clinical flare-up in this udder.

For two or three days there is a very high leucocyte-count. Then the inflammation declines, but the organisms are still there and then the leucocyte-count comes almost to normal.

And so with *Aerobacter aerogenes* infection we may have a chronic mastitis. In the diagnosis of these coliforms one should culture the milk fresh, but also incubate the milk if the fresh milk-samples show no growth.

Question: Prof. D e d i é (Germany):

How much milk should one use to culture the *Aerobacter aerogenes?*

Answer: Prof. S c h a l m (U.S.A.):

In the experimental cases we have learned that we must make cultures of at least one millilitre of milk.

What we do, we have a number of plates with one millilitre, half a millilitre, a tenth of a millilitre and a hundredth of a millilitre. Then we usually isolate a small number of micro-organisms in these high quantities of milk. One must use higher quantities of milk to isolate the micro-organisms.

Remark: Dr. K l a s t r u p (Denmark):

I feel that there are quite more problems about the resistance to masitis besides the low cell-count of the milk.

I have carried out some experimental work of staphylococci-infections of the udder. We infected cows artificially with very small numbers of staphylococci, between 40 and zero; mostly between zero and 5 staphylococci.

The heifers, which we tested before they were infected, were examined on chemical substances of the milk. The milk was also examined on the total cell-counts as well and before infection of these heifers; we were sure that the milk had a normal cell-count and a normal chlorine and glucose-content.

At the end of this experiment the difference of infections could be connected to the pathogenicity of the staphylococci-strains more than to the individual cows. I think that the general condition of the cow is quite important.

We must be careful not to consider only the leucocyte-count of the milk. In my opinion there are many more problems.

Answer: Prof. S c h a l m (U.S.A.):

It would be wrong if the participants of this congress went home with the impression that we must have necessarily leucocytes in the milk, or we are lost if this is not the case.
I am always speaking about the pathogenicity of coliform-strains. At least with our experimental coliform-strains clinical mastitis is partially or completely prevented when we have the leucocytes on the upper normal cell-count, that is to say about 300.000 till 500.000 cells per ml. of milk. This count is not normal, it indicates some irritation of the udder.
In Germany there is some feeling that feeding conditions will lead to coli-mastitis, coming by way of the blood into the udder. I do not know if this is a possibility, but I would say that if the coli comes by way of the blood, it would enter in the basis of the gland rather than down in the teats.
In chronic mastitis my experience indicates that the inflammatory reaction begins in the lower ends of the gland. If we dissect the mammary gland we will find that the inflammatory zône is in the cystern of just about the cystern. I think the coliform-mastitis comes by teat-road and especially those cows with a very low cell-count will get the coliform mastitis.

Question: Dr. E d w a r d s (England):

I should like to ask Prof. S c h a l m: which are predisposing conditions for the *Escherichia coli*-infections in cows?
I remember a visit to the herds of Prof. S c h a l m and we talked about the same problem and I can remember that he told 13 years ago that the Gram-negative organisms were of considerable importance. Up to that time the Gram-negative germs were not important in our country.
I would like to ask him more especially what factors of hygiene and management may be responsible for the occasional infections with *Escherichia coli*. Important was, I think, not the buildings but the floors. In other words do these cows lie on straw or another bedding that may be responsible for the infection of *Escherichia coli?*
It is important in experiments to know if there are some typical serological types. As in other diseases it is important to know with which kind of *Escherichia coli* we are dealing. We know that there are very important serological types of *Escherichia coli* in pig diseases.
I should like to ask Prof. S c h a l m if he was able to find a typical serological type of *Escherichia coli,* which is connected with an infection of the udder.

Answer: Prof. S c h a l m (U.S.A.):

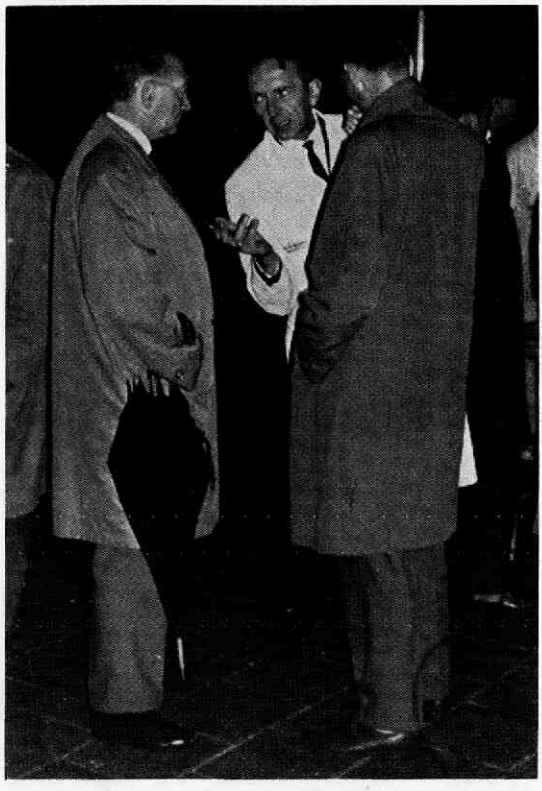
These particular herds Dr. E d w a r d s mentioned, have the most ideal, sanitary and hygienic condition I ever worked with.
The cows are never out on the ground. Thirty cows are within an enclosure, covered with cement that is well bedded with straw and a roof is present to protect them from rain. After each milking the cup-liners are sterilized and between milking of cows the teat-cup are dipped in chlorine. Chlorine we used at the time before the discovery of hibitane. Everything was ideal. Even the control on streptococci and staphylococci mastitis was ideal. In our institute we don't type the different colis.
You cannot type the *aerobacter* serologically. In U.S.A. there are more herds with considerable problems with a coliform mastitis. I may be wrong in this, but I feel that the coliform-mastitis will become much more important in future.

Remark: Prof. D e d i é (Germany).

Prof. S c h a l m talked about coli-mastitis in Germany. We found it in single quarters. Generally the coli-mastitis occurs in one quarter.

Remark: Prof. V a n d e r S c h a a f (The Netherlands):

I should like to tell something about the coliform-mastitis in Holland.
It is now about ten years ago, I think in the early fifties, that my colleague B a u d e t came to Leeuwarden one day with ten cultures of coliforms. I have typed them and it was shown that it was all *Aerobacter aerogenes*. Now we call them *Klebsiella*. That means that in that time *Klebsiella pneumoniae* was present in the province of Gelderland.
In the Clinic for Internal Diseases of the Veterinary Faculty at Utrecht we sometimes find acute coli-mastitis. In many cases the animal will die. And in most cases we found coli together with *Clostridium perfringens*. If we would not have carried out anaerobic culturing of the sample of the milk, we would not have found *Clostridium perfringens*, for this only grows anaërobically.
If there is gas in the udder, if the animal is severely ill, you should always use both culture-methods aërobical and anaërobical, to find out which germs are present in the udder.
We never found merely *Escherichia coli* in the udder from those animals, which were severely ill.



Explanation on the dairy „Campina" at Eindhoven.

Exchange of views.

Critical judgement of wooden shoes.

Mechanical Milking and Mastitis.

by C. H. CAZEMIER*)

Introduction.

Dr. Brus: *Mr. Cazemier, when we started almost two years ago to investigate in cooperation with you and Mr. de Rooy the correlations in management of the dairy farm and mastitis, we were very glad.*
It will always remain interesting to discuss the importance of bacteria on one side and the situation of the udder on the other side.
I think the answer will not be black or white, but grey. It is the task for the investigators in the field to find out how grey it is. It's not possible to do this in the laboratory.
We are interested to learn of your first results.

Introduction.

For many years the technique of milking has been looked at as a factor of importance in the prevention and eradication of mastitis. When antibiotics were discovered and these — how valuable as an aid in mastitis control they may be — had not brought the originally expected progress, even more attention was paid to milking.
The research-work carried out in this field (mechanical milking) up to now, has by no means led to agreement on all points. The main factors which have been put forward can be mentioned as follows:

with respect to milking:

insufficient preparation of the udder before the machine is put on;
too much time between preparation and putting the machine on;
letting the machine work on the udder, after milkflow has already ceased;
taking off in a rough way (under vacuum);
rough and prolonged stripping;

with respect to machine:

vacuumpump with insufficient overcapacity, resulting into an irregular and occasionally too low vacuum;
too high vacuum;
flow of milk away from the teat too slow;
irregularly working pulsator with an insufficient sharp transition from vacuum to atmospheric pressure and vice versa between teat-cup and liner;
teat-cup liners: worn out, not soft enough and elastic enough, hard mouth piece and a too wide bore.

Some of the faults mentioned above are due to the facts that one man has too many milking units at his disposal and to imperfect organization of the work. Practical research and experience especially have drawn attention to these factors.

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Investigations made into the influence of special factors, have often given somewhat contradictory results and have to a large extent not been able to confirm the results of this research work in praxis. In 1961 N e a v e and D o d d declare that there may be a relation between machine milking and mastitis and that the type of teat-cup liner is certainly of importance. They however state that it has not been proved that mastitis outbreaks can be prevented by the single careful use of the milking machine under the application of a certain number of rules, as is maintained by some authors. They consider a well-designed practical investigation to be essential, although this would be difficult to arrange.

As far as the influence of the teat-cup liner mentioned above is concerned it may be said that there is rather a lot of evidence, that the so called moulded liner in general has a less favourable effect on udder health as compared with the type of liner described as extruded liner.

That there is inconsistency between the results based on investigations in praxis, and those reported from the more fundamental research work, is not in our opinion very surprising.

Milking is influenced by the personal character of the milker, a complete standardization of it is actually impossible, as is a real imitation of the great number of types of milking which can be found in praxis. Besides milking, hygiene both during milking and in the stable, is of importance and in praxis can not be dissociated from milking as such. On the other hand most scientific research work is based on a relatively small number of animals, often heifers. Differences in susceptibility between the animals and the average susceptibility in the entire group can under similar conditions, considerably influence the results. We have fully encountered the difficulties of research work in praxis, in a research project on this subject now being carried out in The Netherlands.

Up till now little systematic research in this field has been done in our country. In praxis, by far the most important factor in this respect has always been considered the fact that the cows should be stripped well.

The investigation mentioned above was started in 1962, being carried out in cooperation between the Provincial Animal Health Service in North-Brabant and the Research Institute for Animal Husbandry „Schoonoord" at Zeist. The investigation first included 38 herds, which number was extended later to 57 (approx. 750 cows), nearly all mixed farms situated in the area of the Co-operative Dairy Factories at Dongen and Oosterhout. The herds were classified into two groups on the basis of 13 3-weekly investigations (in canmilk samples) on B.M.R. (Brabantic Mastitis Reaction) from February till September 1962. A B.M.R. of 2 points in a can sample was considered as being a positive reaction. The average percentage of B.M.R.-positive can samples in the entire group during the period mentioned above was 11.65 Also for the entire group the average number of times that one or more cans were positive was 4.28.

Herds in which during that period at least 11% of the cans delivered was positive, were considered to be test-herds. This group included 27 herds with an average percentage of positive can samples of 18.4. The remaining herds were called control-herds. This group consisted of 30 herds, their average percentage of positive can samples was 3.4.

Based on what we know about the physiology of milk ejection and on what is common practice f.i. with respect to pulsation rate and height of

vacuum, one can imagine a method of milking which as much as possible promotes a good and rapid milking of the cow without irritation of the udder tissue. Descriptions of such a method of milking can be found in many publications e.g. in „Machine Milking" (London 1959).
This method of milking has served as a standard for judging the milking technique in the herds included in this investigation. To get an evaluation as objective as possible, time studies were made on all herds. More attention was paid to the degree of having a good idea of the job, than to beauty faults and perfection.

Results

In table I a survey is given of the evaluation of the various factors taken into account, for both groups of herds.
A strict definition of what is exactly meant by „good", „moderate", „bad" etc. is difficult to give. As important points with regard to preparation may be mentioned: cleaning, if necessary, a clear but not rough massage, any pre-milking of a few jets of milk.
With regard to the application of the teat-cups: is the teat clearly led into the chamber, is there a visable turning around of the teat-cup after the milker has moved his hand away; with regard to the removing of the teat-cups: is this or is it not done in a rough manner and/or under vacuum; hand stripping: is this done quickly up to approx. one min., with the whole hand and clearly directed towards removal of the last milk that can be got, from the places where it may be found. Or does it take a long time (more than 3 min.), is it done perhaps with wet hands, more or less rubbing of the teat between thumb and forefinger, without there being clearly looked for the last milk that eventually could be obtained.
Also the evaluation of the organization of the work cannot be described clearly. A logical sequence of the activities is of importance here. If this is lacking, the result is often that the times used and needed for the various activities becomes either too short or mostly too long (waiting time between preparation and putting the machine on, machine milking time, waiting time between taking the teat-cups off and starting hand stripping).
As regards the point: „state of repair of the machine", the quality of the teat-cup liners and the action of the pulsator have especially been taken into consideration.
From the survey given in Table I it can be seen that the factors „change of milkers", „preparation of the cows", „putting the teat-cups on and taking them off", „hand stripping" and the organization of the work (closely connected with the total treatment and machine overmilking time), must be considered as relatively unfavourable for the testherds.
When the total care given to milking and to the milking machine is classified according to the classes „sufficient" and „insufficient" the figures in Table II are obtained. When the same is done for herds which never had a positive sample (10) and for those which for at least 8 of the 13 times had one or more cans positive (12), we obtain the figures given in Table III.

There need be no doubt on the general conclusion that can be drawn from these figures, namely that surely milking on the test-herds is generally done much more carelessly and with less interest.

Tabel I.

	Proefbedrijven (27) *Mastitisherds (Testherds)*			Controlebedrijven (30) *(Controlherds)*		
	Normaal *(normal)*	Te laag *(too low)*	Te hoog *(too high)*	Normaal *(normal)*	Te laag *(too low)*	Te hoog *(too high)*
Vacuum in cm/Hg *(Vacuum in cm/Hg)*	16	2	9	20	2	8
Aantal pulsaties/min. *(Pulsation rate/min.)*	15	1	11	17	2	11
	Goed *(good)*	Matig *(moderate)*	Slecht *(bad)*	Goed (good)	Matig *(moderate)*	Slecht *(bad)*
Voorbehandeling *(Pre-treatment)*	12	9	6	22	6	2
Aanbrengen van de tepelhouders *(Applying of the teat-cup liners)*	14	12	1	23	7	0
Stand van het apparaat *(Position of the appartus)*	17	5	5	23	7	0
Afnemen van de tepelhouders *(Removing of the teat-cup liners)*	17	9	1	25	5	0
Handnamelken *(Hand stripping)*	2	7	15	11	9	5
Machinaal namelken *(Machine stripping)*	0	2	1	0	3	2
Organisatie + verdeling v/h werk *(Organisation & division of work)*	3	6	18	12	10	8
Staat van onderhoud v/d machine *(State of repair of the machine)*	8	10	9	18	9	3

Vervolg tabel I. *Continuation table I.*

Zuig-persslag verhouding *(Pulsation ratio)*	50 — 50 21			70 — 30 of 80 — 20 6			50 — 50 25			70 — 30 of 80 — 20 5		
Wisseling van melkers *(Changing of milkers)*	Normaal *(normal)* 18			Abnormaal *(abnormal)* 9			Normaal *(normal)* 26			Abnormaal *(abnormal)* 4		
Namelken *(Stripping)*	Met de hand *(by hand)* 24			Niet of machinaal *(not or by machine)* 3			Met de hand *(by hand)* 25			Niet of machinaal *(not or by machine)* 5		
Totale behandelingstijd *(Total time of treatment)*	Binnen 9 min. *(within 9 min.)* 11			Langer dan 9 min. *(more than 9 min.)* 16			Binnen 9 min. *(within 9 min.)* 21			Langer dan 9 min. *(more than 9 min.)* 9		
Machine overmelktijden *(Machine over milking time)*	Sporadisch *(seldom)* ± 16			Veelvuldig *(frequently)* ± 11			Sporadisch *(seldom)* ± 25			Veelvuldig *(frequently)* ± 5		
Werkmethode *(Work method)*	P_1A_1 18	P_2A_2 2	P_1A_2 5	P_3A_2 2	P_2A_1 0	P_1A_1 17	P_2A_2 6	P_1A_2 6		P_3A_2 0	P_2A_1 1	
Type melkmachine *(Type of milking machine)*	Hangend *(hanging)* 19			Staand *(standing)* 8			Hangend *(hanging)* 19			Staand *(standing)* 11		
Type tepelvoering *(Type of liner)*	Platte kop *(flat cup)* 14		Bolkop *(spherical cup)* 5	Platte kop *(flat cup)* 1		Bolkop *(spherical cup)* 7	Platte kop *(flat cup)* 16		Bolkop *(spherical cup)* 3	Platte kop *(flat cup)* 7		Bolkop *(spherical cup)* 4	

Table I.

Tabel II.
Table II.

	Proefbedrijven *(Testherds)*	Controlebedrijven *(Controleherds)*
Melken voldoende (Milking sufficient)	7	20
Melken onvoldoende *(Milking sufficient)*	20	10

Tabel III.
Table III.

	10 bedrijven, nimmer busmonster positief B.M.R. *(10 herds, never positive B.M.R.-canmilk samples)*	12 bedrijven, minstens 8x busmonsters positief B.M.R. *(12 herds, at least 8 times a positive B.M.R. canmilk sample)*
Melken voldoende *(Milking sufficient)*	7	1
Melken onvoldoende *(Milking insufficient)*	3	11

This conclusion is confirmed by the figures in Table IV, where a survey is given of the results of milk quality analysis in both groups of herds from January to September 1962.

Tabel IV.
Overzicht van de percentages bedrijven met afwijkingen bij het kwaliteitsonderzoek van de melk (gemiddelde over 21 onderzoekingen).
Table IV.
Survey of the percentages of herds with deviating quality of the mi k (average of 21 investigations).

Bedrijven met weinig mastitis (30) *(Herds with few cases of mastitis (30)*	Bedrijven met veel mastitis (27) *(Herds with many cases of mastitis (27)*
Gem. % bedrijven met reductase 2 8.3 *(Average % herds with reductase 2)*	10.9
Gem. % bedrijven met reductase 3 4.3 *(Average % herds with reductase 3)*	8.9
Gem. % bedrijven met reinheid 2 8.4 *(Average % herds with cleaness 2)*	17.7
Gem. % bedrijven met reinheid 3 0.6 *(Average % herds with cleaness 3)*	3.5

At the same time however these figures underline the question whether the less care given to milking directly and as such on the test-herds or the

less hygienic way of working closely connected to this, will be primarily responsible for the higher degree of mastitis found in these herds.
Some indication in this direction might be obtained by considering the results of quality analysis for the classes „sufficient" and „insufficient milking", separately, as is done in Table V.

Tabel V.
Overzicht van de percentages bedrijven met afwijkingen bij het kwaliteitsonderzoek van de melk (gemiddeld over 21 onderzoekingen).

Table V.
Survey of the percentages of herds with deviating quality of the milk (average of 21 investigations).

	Bedrijven met weinig mastitis *(Herds with few cases of mastitis)*		Bedrijven met veel mastitis *(Herds with many cases of mastitis)*	
	melken voldoende *(milking sufficient)* (20)	melken onvoldoende *(milking insufficient)* (10)	melken voldoende *(milking sufficient)* (7)	melken onvoldoende *(milking insufficient)* (20)
Gem. % bedrijven met reductase 2 *(Average % herds with reductase 2)*	7.6	10.—	8.2	11.9
Gem. % bedrijven met reductase 3 *(Average % herds with reductase 3)*	4.3	4.3	8.8	9.3
Gem. % bedrijven met reinheid 2 *(Average % herds with cleaness 2)*	7.6	10.—	15.—	18.8
Gem. % bedrijven met reinheid 3 *(Average % herds with cleaness 3)*	0.7	0.5	2.7	3.8

From these figures it can be seen that in the two groups where milking was considered to be sufficient, the average quality and especially the purity of the milk is less favourable in herds with more mastitis. The same difference is observed on the herds on which milking was characterized as insufficient. Within the two groups of herds as a whole (test-and control-group resp.), there is, between the classes „milking sufficient" and „insufficient" no great difference in the quality of the milk, although in both cases the quality is somewhat inferior in the class „milking insufficient". It should be recognized especially that on the testherds classified in the group „milking sufficient", the quality of the milk seems to be inferior than on the control-herds classified as „milking insufficient".

Tabel VI.

Uitkomsten kwartiermonster onderzoek, bacteriologisch en op B.M.R. naar proef- en controlebedrijven, over 3 onderzoekingen in de periode juli t.m. september 1962.

Table VI.

Results of bacteriological and B.M.R. research of quarter samples. Data of 3 investigations from test- and controlherds during the period July - September 1962.

	1e onderzoek *1st investigation*		2e onderzoek *2nd investigation*		3e onderzoek *3rd investigation*	
	Proef-bedrijven *(Test-herds)*	Controle-bedrijven *(Control-herds)*	Proef-bedrijven *(Test-herds)*	Controle-bedrijven *(Control-herds)*	Proef-bedrijven *(Test-herds)*	Controle-bedrijven *(Control-herds)*
Aantal bedrijven *(Number of herds)*	25	13	25	13	25	13
Aantal koeien *(Number of cows)*	367	186	345	179	324	175
Aantal kwartieren *(Number of quarters)*	1460	743	1372	716	1288	699
% kwartieren pos. B.M.R. ($\geqq 3+$) *(% of quarters positive B.M.R. ($\geqq 3+$))*	16,8	8,1	15,2	9,1	16,1	7,2
% kwartieren met *Strept. agalactiae (% of quarters with Strept. agalactiae)*	10,3	2,8	11,4	3,8	12,9	4,6
% kwartieren met *Staphyloc.* B.M.R. $\geqq 3+$ *(% of quarters with Staphyloc. B.M.R. $\geqq 3+$)*	3,5	2,2	2,8	2,4	3,3	2,0
% kwartieren met *Staphyloc.* B.M.R. $<3+$ *(% of quarters with Staphyloc. B.M.R. $<3+$)*	4,3	5,00	7,9	8,0	11,7	13,9
% kwartieren *Strept. dysgalactiae (% of quarters Strept. dysgalactiae)*	1,9	2,2	1,5	0,8	1,1	0,9
% kwartieren negatief *(% of quarters negative)*	79,9	87,4	76,0	85,1	71,0	78,7
% koeien *Strept. agalactiae (% of cows Strept. agalactiae)*	24,8	8,6	22,0	8,9	30,8	13,7
% koeien *Staphyloc. (% of cows Staphyloc.)*	16,6	16,1	19,1	20,1	24,1	22,9
% koeien *Strept. dysgalactiae (% of cows Strept. dysgalactiae)*	4,6	4,3	4,6	1,1	2,4	1,7
% koeien positief B.M.R. ($\geqq 3+$) *(% of cows positive B.M.R. ($\geqq 3+$))*	41,4	22,6	37,7	25,1	38,3	20,6
% koeien negatief (bacteriologisch) *(% of cows negative (bacteriological))*	53,7	70,4	53,9	69,8	42,6	61,7

Tabel VII.

De mate van optreden van nieuwe infecties. Aantal koedagen per nieuwe infectie (in kwartieren) of positieve reactie op B.M.R.

Table VII.

Rate of new infections. Number of cow-days per new infection (in quarters) or positive reaction on B.M.R.

	Proefbedrijven *(Testherds)*	Controlebedrijven *(Controlherds)*
Strept. agalactiae	173	424
Strept. dysgalactiae	1708	
Staphyloc., tevens *(together with)* B.M.R. $\geqq$ 3+	326	598
Staphyloc., tevens *(together with)* B.M.R. $<$ 3+	107	111
B.M.R. $\geqq$ 3+	102	178

Tabel VIII.

Reactie percentage in kwartieren, waarin bij een voorgaand onderzoek geen enkele reactie of besmetting werd gevonden, resp. reactiepercentages in kwartieren, waarin bij een voorgaand onderzoek uitsluitend een positieve B.M.R. werd gevonden.

Table VIII.

Percentages of reaction in quarters, which were negative in the foregoing sampling, respectively percentages of reaction in quarters, which in the foregoing sampling were only positive on B.M.R.

	Proefbedrijven *(Testherds)*		Controlebedrijven *(Controlherds)*	
	1874 vrije kwartieren *(negative quarters)*	141 B.M.R. pos. kwartieren *(B.M.R. positive quarters)*	1203 vrije kwartieren *(negative quarters)*	62 B.M.R. pos. kwartieren *(B.M.R. positive quarters)*
B.M.R. ($\geqq$3+) + *Strept. agalactiae*	1,8	12,—	0,4	3,2
B.M.R. ($\geqq$3+)	2,9	22,—	2,8	12,9
B.M.R. ($\geqq$3+) + andere besmetting (infection non *Strept. agalactiae*)	1,8	11,3	0,8	3,2
Strept. agalactiae	1,8	5,8	1,2	4,9
Andere besmetting (infection non *Strept. agalactiae)*	8,1	9,9	7,0	27,4
Vrij *(Negative)*	83,6	39,—	87,8	48,4

Tabel IX.

Reactie percentages in kwartieren waarin bij een voorgaand onderzoek uitsluitend Strept. agalactiae werd gevonden.

Table IX.

Percentages of reaction in quarters which in foregoing sampling were only positive on Strept. agalactiae.

	Proefbedrijven *(testherds* 123 kwartieren *(quarters)*	Controle bedrijven *(Controlherds)* 33 kwartieren *(quarters)*
B.M.R. (≧3+) + *Strept. agalactiae*	31,7	24,3
B.M.R. (≧3+)	2,5	0,0
B.M.R. (≧3+) + andere besmetting (infection non *Strept. agalactiae)*	1,6	3,0
Strept agalactiae	31,7	21,2
Andere besmetting (infection non *Strept. agalactiae*)	6,5	9,1
Vrij *(Negative)*	26	42,4

These observations in our opinion give some support to the idea that at least in this case, if more mastitis results from bad milking techniques, it are in the first place the bad hygienic conditions closely connected with these bad techniques, that must be regarded for as being responsible.

Interesting also in this connection are some of the figures than can be obtained from the investigations into the bacteriological picture and the B.M.R. of quarters.

About once every 4 or 5 weeks the quarter samples of all cows are examined bacteriologically and on B.M.R. In Table VI a survey is given of the first three samplings (July to September 1962). As the figures reveal, during this period this part of the investigation was restricted to a smaller number of herds. Especially the degree of *Streptococcus agalactiae* infection appears to be much higher in the test-herds than in the control-herds.

During the three tests, 50—60% of the cows on the test-herds showed one or more infected quarters. In the control-herds this percentage was 30—40%. In Table VII the rate of new infections in quarters is given based on the number of cow days in both groups. Also the rate of new positive reactions on B.M.R. (3+) in quarters is given.

In the test-herds there is a definitely higher frequency of new infections and of newly B.M.R. positive quarters than in the control-herds. Some doubt may be put to the significance of a Staphylococci-infection, not accompanied by a positive reaction on B.M.R. Based on what is said before the question arises as to what may be the reason for the foregoing higher number of new B.M.R.-positive quarters in the test-herds. The inferior milking technique as such with consequently a higher degree of irritation of udder tissues, or the less hygienic way of milking resulting from it and hence a higher degree of contamination.

Table VIII summarizes the reactions found in quarters in which nothing whatever resp. only a positive B.M.R. was found in the foregoing sampling. In few words: what happens to quarters now found to be negative in all respects, between the present and the following sampling.
The figures given show that starting from these negative quarters, the rate of newly B.M.R.-positive quarters is practically the same in test-herds as in control-herds. Indeed the total number of newly B.M.R.-positive quarters is relatively higher in the test-herds, but this difference is caused by quarters in which at the same time one of another infection has been established. This difference has been proved to be statistically highly significant. Thus in our opinion these data once again lend support to the idea that at least in our group of herds, as far as bad milking techniques have an unfavourable influence on udder health, this is mainly caused by the higher degree of contamination that in practice, will nearly always be closely connected with bad milking techniques.
As an interesting feature as such it can be seen from these figures that as well in test- as in control-herds, B.M.R.-positive quarters are more likely to get infected later on, than are negative quarters.

In Table IX it is shown that in the test-herds quarters positive on *Strept. agalactiae* are somewhat (not significant) more likely to get a positive B.M.R., than in the control-herds. Here one could think of an influence of milking techniques, but also on the fact that contaminations will be more frequently and heavier in the test-herds.

In summary, according the literature, under good milking conditions there still may be an influence from the type of teat-cup liner on udder health. Also according to the literature, where inferior milking techniques are employed in practice, an unstable udder health may in general be one of the results. This conclusion is supported by the data presented in this paper.
At the same time some evidence is given that it may be not the bad milking technique as such that causes the higher degree of mastitis, but more the enlarged chances of contamination, by which in practice bad milking techniques are very often accompanied.

SAMENVATTING

Sedert lang wordt de melk-techniek, speciaal van het machinale melken, als een belangrijke factor gezien bij de preventie en bestrijding van mastitis.
In praktijk-onderzoek werd hoofdzakelijk aandacht besteed aan de volgende punten:

A. Met betrekking tot de melk-techniek:
   1. voorbehandeling;
   2. tijd tussen de voorbehandeling en het moment van aansluiten van de machine;
   3. blind melken;
   4. methode van aansluiten en afhalen van de tepelbekers;
   5. ruw en langdurig strippen.

B. Met betrekking tot de machine:
   1. vacuum-pomp: eventueel onvoldoende overcapaciteit, waardoor een regelmatig en soms een te laag vacuum ontstaat;
   2. vacuum-pomp: vacuum meten, eventueel te laag vacuum;
   3. te langzaam afvloeien van de melk uit de bekers;
   4. pulsator: eventueel onregelmatig of een onvoldoende scherpe overgang tussen zuig en pers onderbrekingsslag;

5. tepelvoeringen-dikbuikig, te hard, te weinig elastisch, stugge kop, te wijde boring etc.

De kwartier melkmonsters werden bacteriologisch en met behulp van de Brabantse Mastitis Reactie (B.M.R.) onderzocht door de Provinciale Gezondheidsdienst voor Dieren te Boxtel.

Op grond van het B.M.R.-onderzoek van de busmelkmonsters van de zuivelfabrieken Dongen en Oosterhout, dat elke 4—5 weken plaats vond, werden twee groepen bedrijven uitgezocht nl. de groep van de positieve-bedrijven en die van de negatieve bedrijven, in de tabellen resp. proefbedrijven en controlebedrijven genoemd. De positieve bedrijven hadden in de eerste periode van de proef steeds een positief B.M.R.-onderzoek van de busmelkmonsters, terwijl de negatieve bedrijven in de eerste periode van de proef steeds of vrijwel steeds een negatief B.M.R.-onderzoek van de busmelkmonsters haalden.

In tabel I wordt een overzicht gegeven van een aantal waarnemingen. De waardering „goed", „matig" of „slecht" zijn moeilijk exact te omschrijven, evenals een aantal andere begrippen in tabel I genoemd. Uit tabel I blijkt, dat op de positieve bedrijven in verschillende opzichten zowel wat de melktechniek, de staat van onderhoud van de machine, als de organisatie van het werk betreft, minder goed zijn dan de negetieve-bedrijven.

Dit komt nog duidelijker tot uitdrukking in de tabellen II en III.

Dat ten gevolge van het minder accuraat melken op de positieve bedrijven de kwaliteit van de melk zoals deze nu wordt bepaald, minder is dan die van de negatieve bedrijven, is duidelijk te lezen uit de tabellen IV en V.

In tabel VI wordt een overzicht gegeven van de eerste 3 melk-onderzoekingen. Opmerkelijk is het verschil in percentage *Str. agalactiae*-infecties op de positieve en de negatieve bedrijven. Het perecentage kwartieren met stafylokokkeninfecties is ongeveer gelijk.

Tabel VII geeft aan dat er op de positieve bedrijven een hogere frequentie is betreffende het ontstaan van nieuwe infecties of nieuwe positieve B.M.R.-uitslagen dan op de negatieve-bedrijven. Deze grootheden zijn uitgedrukt in koe-dagen per infectie of per positieve B.M.R.

Samenvattend kan worden gesteld dat de melktechniek in het algemeen van groot belang is bij het ontstaan of voorkomen van uierontsteking bij het rund. Daar, waar slecht wordt gemolken, ontstaan meer uierontstekingen.

Over het algemeen is de kwaliteit van de melk op bedrijven waar slecht wordt gemolken, minder dan op bedrijven waar goed wordt gemolken.

Discussion

following the lecture of Ir. C a z e m i e r.

Question: Mr. D i j k s t r a (Leeuwarden, The Netherlands):
Is there some influence of blind milking on the occurrence of mastitis?

Answer: Ir. C a z e m i e r (Zeist, The Netherlands):
The overmilking-time is commoner in the test-herds than in the control-herds, however this difference is not very great.

Question: Dr. E d w a r d s (England):

1. It is very difficult to understand one thing, a very important thing. What is the meaning of control- and test herds here?
   Test-herds; that means you have done something and the control-herds you have done nothing? That is the first question.
2. And next, what is the significant difference between the control-herds and the test-herds, applied to your difference here? Formerly — I

think — you said the machine wat not so important as hygiene. I think that is a very good summary.

3. And the matter of overmilking I think is emphasized by you. I think I agree with many workers who are here, that overmilking is the most dangerous of machine milking.

Answer: Ir. C a z e m i e r (The Netherlands):

As to the question of test-herds and control-herds, we must have two names for the two groups. The test-herds are the herds which have more mastitis than the control-herds according to the B.M.R. The control herds have less mastitis according to the B.M.R. It was only a matter of name. An especial test has not be done with the test-herds in relation to the control-herds, up to now at least. The test-herds have a high percentage of positive B.M.R..

This division in two groups is not a very sharp one, but based on the data we had, it was the best one to be reached.

And now the question of overmilking time. Of course we cannot judge the influence of special factors in such a research. There are a number of factors which influence the coming into existence of mastitis. In literature some experiments are described which give a clear difference between normal milking and overtime milking, but there are other experiments in which the difference between normal time milking and overmilking-time concerning mastitis is not clear. I remember the reports of N e a v e and D o d d in Kopenhagen last year. There was a combination of high vacuum and overmilking time.

So two factors, namely a vacuum of about 50 centimeters and an overmilking time of 5 minutes. N e a v e and D o d d could not find a great difference between these two groups of herds, although in the groups of overmilking time and high vacuum there were more lesions on the teats.

Remark: Prof. D e d i é (Germany):

I agree with the speaker that bad-milking is mostly combined with a bad milker and so quite a lot of different troubles fall together.

It is quite interesting to watch what happens if by a good milker and good hygienic conditions some or more changes are made in the milking technique. For instance we have had a good milker and we had good hygienic conditions, but the machine milking was changed by a pipeline-condition with too high a vacuum and prolonged milking time. The result in fact was mastitis.

So I think it is very clear from this example that a bad milking technique can be the reason of many troubles.

Question: Dr. K l a s t r u p (Denmark):

In both groups, the test-group and control-group, there are different herds with a vacuum which was too high. I wonder you did not compare the incidence of infections from especially staphylococci or the incidence of mastitis in general or the incidence of B.M.R. between the herds with a high vacuum and the herds with a normal vacuum.

Answer: Ir. C a z e m i e r (The Netherlands):

Up till now we have not done so. The vacuum which was too high was more than 40 c.m. Hg. and the vacuum which was too low was less than 30 cm. Hg. The vacuum should be normally between 35 and 37 cm. Hg. Maybe there will be some difference between the different groups as you suppose. We will try to find it out.

I thank you for the suggestion.

Über die Möglichkeiten und Grenzen eines Eutergesundheitsdienstes.

von A. SCHEINER*)

Einleitung.

Dr. Brus: *Unserer Dienst hat mit dem Gesundheitsamt in Hannover schon sehr viel über Mastitis und Mastitis-Bekämpfung diskutiert. Wir wissen dass Sie speziell in der Diagnostik viel Erfahrung haben. Es ist mir darum eine ganz besondere Freude Sie, Dr. Scheiner, jetzt zu Ihrem Vortrag bitten zu dürfen.*

Vor einigen Tagen hat das Tiergesundheitsamt Hannover sein 50-jähriges Jubiläum gefeiert und rückblickend allen in der Land- und Milchwirtschaft tätigen Personen aufgezeigt, was in diesen 50 Jahren an wissenschaftlichen Arbeiten und tierärztlichen Untersuchungen geleistet worden ist. In den weit umfassenden Rahmen dieser wissenschaftlichen Arbeiten und tierärztlichen Untersuchungen fallen zu einem sehr gewichtigen Teil die Euter- und Milchuntersuchungen.

Mit diesen Euter- und Milchuntersuchungen hat sich das Tiergesundheitsamt sehr eingehend befasst. Dabei kam ihm sehr zustatten, dass es stets einen engen Kontakt zu den praktizierenden Tierärzten und zu den Bauern hatte. Es konnte die Untersuchungen nicht nur im Institut, sondern in der Hauptsache draussen im Stall an der Kuh vornehmen und überprüfen.

Bedeutende Arbeiten über die Feststellung der Rinderbrucellose mit Hilfe der Milchuntersuchungen sind von Prof. Karsten im Tiergesundheitsamt durchgeführt worden. Aber auch an der Erforschung, Ermittlung und Bekämpfung des gelben Galtes ist vor allem von Dr. Ehrlich, dem späteren Direktor der Tiergesundheitsämter Stettin und Münster, gearbeitet worden. Das schwarze Seihtuch nach Ehrlich für die Vormelkprobe und die nach Ehrlich modifizierte Schnellkatalaseprobe sind feste Begriffe in der Geschichte der Milchhygiene geworden.

Ehrlich hat seine Erfahrungen und Kenntnisse bei den Milchuntersuchungen und in der Bekämpfung der Euterkrankheiten vor allem in Vorzugsmilchbeständen erworben, die in den 20-iger Jahren hauptsächlich mit seiner Hilfe und Beratung aufgebaut und überwacht wurden. Bis auf den heutigen Tag bestehen diese Vorzugsmilchbetriebe in Deutschland, die eine rohe Milch bester Qualität in den Handel bringen.

Für diese Vorzugsmilchbetriebe führte Ehrlich eine freiwillige, tierärztliche Milch- und Stallkontrolle ein und arbeitete Überwachungs- und Untersuchungsverfahren aus, die von den heute gültigen, gesetzlichen Vorschriften für die tierärztlichen Untersuchungen in Vorzugsmilchbeständen kaum abweichen.

Nach den heute bestehenden gesetzlichen Vorschriften werden die Vor-

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zugsmilchbestände monatlich, also 12 x im Jahr, auf Euterkrankheiten durch den beamteten Kreistierarzt (Veterinärrat) kontrolliert. Er lässt, nachdem er die Euter untersucht hat, unter seiner Aufsicht Milchproben von jeder einzelnen Kuh entnehmen und schickt diese an das Tiergesundheitsamt. Die eingesandten Milchproben werden auf alle milchhygienisch wichtigen Krankheiten zytologisch, bakteriologisch, serologisch und durch den Tierversuch geprüft.
Da diese Vorzugsmilchbetriebe von Anfang an tierärztlich untersucht worden sind, und sie auch bis auf den heutigen Tag eine Trinkmilch bester Qualität in den Verkehr bringen, sind sie immer wieder von namhaften Milchhygienikern als ein Musterbeispiel hingestellt worden, wie Trinkmilch bester Qualität erzeugt werden kann. Ähnlich wie in diesen wenigen Vorzugsmilchbetrieben — im Bereich des Tiergesundheitsamtes sind es etwa 50 Betriebe mit etwa 1.000 Kühen — wollte man eine tierärztliche Milch- und Stallkontrolle auch in den übrigen milcherzeugenden Betrieben einrichten.

Für diese Betriebe, die ihre Milch zur Pasteurisierung an Molkereien lieferten, war aber im Deutschen Milchgesetz eine regelmässige tierärztliche Überwachung der Milchkühe nicht vorgesehen. Schon 1925 hatte E h r l i c h für diese Betriebe ein freiwilliges Streptokokken-Bekämpfungsverfahren eingeführt. Regelmässig konnten vierteljährlich Einzelmilchproben aller Kühe eines Bestandes mikroskopisch auf Milcheiter und Streptokokken im Tiergesundheitsamt untersucht werden. Der Besitzer und der Tierarzt erhielten eine Abschrift des Untersuchungsbefundes. Bei festgestellter Erkrankung sollte der Besitzer die Tiere absondern und nach tierärztlicher Anweisung behandeln.
Diesem Verfahren konnten sich die Besitzer einzeln oder über ihre Molkerei anschliessen. Ausser einer Reihe von Einzelanschlüssen, meist grössere Betriebe, die die Arbeit ihrer Melker kontrollieren wollten, schlossen sich 3 grosse Molkereien mit allen milchliefernden Betrieben diesem Überwachungsverfahren an. Jedoch führte nur eine Molkerei die Untersuchungen 4 x im Jahr durch, während die beiden anderen Molkereien sie 2 x im Jahr durchführten.
Die praktizierenden Tierärzte oder Vertrauenstierärzte des Tiergesundheitsamtes suchten die Betriebe auf und prüften mit einer schwarzen Milchprüfschale die Milch aus jedem einzelnen Viertel. Nur beim Verdacht einer Eutererkrankung wurde eine Milchprobe aus dem betreffenden Viertel und eine Milchprobe aus den anderen 3 Vierteln dem Tiergesundheitamt zur mikroskopischen Untersuchung eingeschickt. Dieses freiwillige Untersuchungsverfahren zeigte aber gleich zu Beginn Mängel. Von den Tierärzten wurden die Untersuchungstermine in den seltensten Fällen innegehalten. Vor allem nicht in den Sommermonaten, wenn die Kühe auf der Weide waren. Ausserdem wurde von ihnen regelmässig eine grosse Anzahl kleiner und mittlerer Betriebe kontrolliert, in denen sie nie Eutererkrankungen feststellten. Daher empfanden sie und die betreffenden Bauern diese durchgeführten Kontrollen als überflüssig und lästig.
Durch den Krieg wurde dieses freiwillige Streptokokken-Bekämpfungsverfahren zwangsläufig wieder eingestellt. Immerhin sind vor dem Kriege jährlich etwa 100.000 Milchproben im Tiergesundheitsamt untersucht worden.

1950 wurde erneut im Tiergesundheitsamt Hannover mit der Einrichtung eines Euterüberwachungsverfahrens begonnen. Dieser neue Eutergesundheitsdienst wurde so aufgebaut, wie ihn das Tiergesundheitsamt in Kiel eingerichtet hatte.

Auf freiwilliger Basis konnten sich ihm wieder die Besitzer einzeln oder über ihren Milchkontrollverein oder ihre Molkerei anschliessen. Von einer generellen tierärztlichen Euteruntersuchung wurde aus den oben angeführten Mängeln und aus personellen und materiellen Gründen Abstand genommen. Die Tierärzte sollten erst nach erfolgter Feststellung von Euterkrankheiten hinzugezogen werden. Daher wurde mit der Entnahme und Einsendung der Einzelmilchproben das Kontrollpersonal der Milchkontrollvereine und Molkereien beauftragt. Sie schickten 2 x im Jahr Einzelmilchproben von jeder Kuh des Bestandes zur Untersuchung auf Sekretionsstörungen ein.

Die Milchproben wurden im Tiergesundheitsamt nach dem Sedimentierverfahren beurteilt und nur die verdächtigen Milchproben auf Erreger von Euterentzündungen untersucht.

Beim Sedimentierverfahren werden 10 ccm Milch 15 Minuten bei 3.500 Umdrehungen zentrifugiert. Anschliessend wird das Sediment beurteilt und nur bei verdächtigem Sediment eine mikroskopische und kulturelle Untersuchung (TKT-Agar, Blutagar, Traubenzuckerbouillon) auf Euterkrankheiten und ihre Erreger durchgeführt.

Mit Hilfe der Abortus-Bang-Ringprobe wurden die Milchproben ausserdem auch auf Brucellose und anschliessend ebenfalls nur die verdächtigen und positiven Proben mit der Milchserum-Langsamagglutination nachuntersucht. Sämtliche Milchproben wurden in Gruppen von 10—20 Milchproben auf Meerschweinchen verimpft, um auch Kühe mit Eutertuberkulose zu ermitteln.

Es wurden also mit diesem neuen Eutergesundheitsdienst die Tierbesitzer über 3 verschiedene Krankheiten informiert, die in ihren Beständen vorkommen konnten:

über die Eutertuberkulose,
über die Brucellose der Rinder und
über die übrigen Euterkrankheiten.

Da das Tiergesundheitsamt in den ersten Jahren nach der Währungsreform noch nicht über genügend Arbeitsraum verfügte, konnte sich der Eutergesundheitsdienst trotz regen Interesses der Molkereien nur ganz allmählich erweitern. Erst 1957 war es möglich, im Hinblick auf die Trinkmilchverordnung Niedersachsens sämtliche Trinkmilch-liefernden Molkereien anzuschliessen. Da aber das Tiergesundheitsamt gleichzeitig auch mit der Untersuchung der Milch- und Blutproben im Rahmen des freiwilligen staatlich geförderten Brucellose-Bekämpfungsverfahren betraut worden war, konnte der grösste Teil dieser Molkereien nur über die Untersuchung von Kannenmilchproben dem Tiergesundheitsamt angeschlossen werden. Sowohl die Kannenmilchuntersuchungen als die Einzelmilchuntersuchungen des Eutergesundheitsdienstes wurden so durchgeführt, dass sie jederzeit auch für die vorgeschriebenen Brucelloseuntersuchungen im Rahmen des Brucellose-Bekämpfungsverfahrens mit ausgewertet werden konnten.

Die Untersuchung von Kannenmilchproben im Rahmen des Eutergesundheitsdienstes sollte allerdings nur eine Notlösung sein. Jedoch wurden wir in der folgenden Zeit davon überrascht, dass vor allem auch die Molkereien und die Oberkontrollassistenten Gefallen an diesem leicht durch-

zuführenden Verfahren bekamen und auch Molkereien, die vorher die Einzelmilchuntersuchungen in ihren Einzugsgebieten durchgeführt hatten, nun ebenfalls zur Kannenmilchuntersuchung übergingen. So waren z.B. 1955 119 Molkereien durch Einzelmilchuntersuchungen dem Tiergesundheitsamt angeschlossen. 1963 sind es nur noch 77 Molkereien, während 117 Molkereien über die Untersuchung von Kannenmilchproben dem Eutergesundheitsdienst angeschlossen sind.
Von Anfang an waren wir im Tiergesundheitsamt mit dieser Entwicklung nicht einverstanden, da die Untersuchung der Einzelmilchproben viel genauere Ergebnisse lieferte als die Untersuchung der Kannenmilchproben mit Hilfe des Sedimentierverfahrens.
Auf der anderen Seite waren aber die Entnahme und Einsendungen von Einzelmilchproben aus sämtlichen Beständen und ihre Untersuchung aus Personalmangel in den Molkereien und in den Instituten nicht durchführbar. Wir hatten auch statistisch festgestellt, dass die Probenehmer in jedem Jahr etwa 90% der Bestände umsonst aufgesucht und hier die Proben entnommen hatten, da Jahr für Jahr bei nur etwa 5—10% der Bestände Sekretionsstörungen festgestellt wurden. Dieser Leerlauf bei der Probe-entnahme und bei der Untersuchung im Institut hat uns viel beschäftigt.

Nunmehr stehen wir mit unserem Eutergesundheitsdienst an einer Wende. Personalmangel in den Molkereien und in den Instituten zwingen zu einer neuen Lösung; ebenso die z. T. unbefriedigenden Ergebnisse der Kannenmilchuntersuchungen mit Hilfe der Sedimentier- und Mikroskopiermethode. Die Vorwürfe, die verschiedentlich geäussert worden sind, dass dieser Eutergesundheitsdienst nur ein Feststellungsverfahren sei, zwingen ebenfalls dazu, uns auch mit dem Einbau einer gezielten tierärztlichen Überwachung und Sanierung der an Mastitis erkrankten Bestände zu befassen.

Ausschauhaltend nach einer anderen Möglichkeit der Durchführung eines Eutergesundheitsdienstes haben wir festgestellt, dass es in Deutschland sehr unterschiedliche Eutergesundheitsdienste gibt:

1. Die monatliche tierärztliche Untersuchung der Euter in Vorzugsmilchbeständen. Einsendung von Einzelmilch- oder Gruppenmilchproben.
2. Eine vierteljährliche tierärztliche Untersuchung der Euter in Markenmilchbeständen. Einsendung von Einzelmilchproben.
3. 2 mal im Jahr durchgeführte tierärztliche Untersuchung der Euter in Markenmilchbeständen und versuchsweise in einem politischen Kreis. Einsendung von Einzelmilchproben aller milchgebenden Kühe oder nur der klinisch verdächtig erscheinenden Kühe.

Alle bis jetzt genannten tierärztlichen Eutergesundheitsdienste kamen nur bei einem geringen Teil der milchliefernden Bestände zur Durchführung. Bei sämtlichen milchlieferden Beständen oder nur bei den Trinkmilchliefernden Beständen der Molkereien oder bei Mitgliedern von Herdbuchgesellschaften werden durchgeführt:

4. Einsendung von Einzelmilchproben 2mal im Jahr durch Probenehmer, Hinzuziehung des Tierarztes nur zur Behandlung erkrankter Kühe.

5. Einsendung von Einzelmilchproben 1mal im Jahr, Einsendung von kannenmilchproben einmal im Jahr. Die Einsendung erfolgt durch Probenehmer.
6. Einsendung von Kannenmilchproben 2mal im Jahr durch Probenehmer.
7. Untersuchung der Milch an der Kuh durch Kontrollassistenten mit Hilfe der schwarzen Schale, der Katalase-Thybromolprobe oder des Indikatorpapiers. Einsendung von Milchproben nur in Verdachtsfällen. Hinzuziehung des Tierarztes auch in diesen letzt genannten Eutergesundheidtsdiensten nur zur Behandlung der erkrankten Kühe.

Daraus ist zu ersehen, das es keine Einheitlichkeit in der Durchführung des Eutergesundheitsdienstes und auch keine Einheitlichkeit in der Erkennung und Bekämpfung der Euterkrankheiten gibt.

Jedem dieser Eutergesundheitsdienste sind aber Grenzen gesetzt.
Bei den zu untersuchenden Vorzugsmilchbeständen, die monatlich untersucht werden müssen, ist es die begrenzte Anzahl der Bestände.
Bei den zu untersuchenden Markenmilchbeständen ist es ebenfalls die begrenzte Anzahl der Bestände, da für eine viertel- bzw. halbjährliche Überprüfung der gesamten milchliefernden Bestände die vorhandenen Tierärzte nicht ausreichen würden, um diese Untersuchungen durchführen zu können.
Einer solchen allgemeinen tierärztlichen Euterüberwachung wären aber auch im Hinblick auf den Milchpreis und die Honorierung der Tierärzte Grenzen gesetzt.
Grenzen sind aber auch einem allgemeinen Eutergesundheitsdienst gesetzt, der sich auf die Einsendung von Einzelmilchproben durch Probenehmer und deren Untersuchung in einem Institut beschränkt und nur in den Fällen, wo Sekretionsstörungen in Beständen gefunden werden, den Tierarzt einsetzt; denn leider stehen uns z. Zt. die erforderlichen Probenehmer auch nicht mehr so ohne weiteres zur Verfügung. Personalmangel und erhöhte geldliche Forderungen erschweren auch diesen eingeengten Eutergesundheitsdienst.
Grenzen sind aber auch einem allgemeinen Eutergesundheitsdienst gesetzt, wenn man ganz klar und nüchtern überrechnet, wieviel Milchproben ein Institut täglich untersuchen kann. So müssten z.B. bei einer 2mal im Jahr erfolgenden Einsendung von Einzelmilchproben aller milchgebenden Kühe vom Tiergesundheitsamt Hannover 1.200.000 Proben untersucht werden. Das wären rund gerechnet 5.000 Proben pro Tag, wobei man bedenken muss, dass aber die Einsendungen fast ausschliesslich in den Wintermonaten erfolgen, somit also in diesen Monaten etwa 8. - 10.000 Proben pro Tag untersucht werden müssten. Hier taucht dann immer wieder der Gedanke auf, ob dieser Riesenaufwand wirklich erforderlich ist, wenn bei den Untersuchungen Jahr für Jahr nur in etwa 10% der Bestände Sekretionsstörungen festgestellt werden.

Idealzustand wäre selbstverständlich eine monatliche Untersuchung von Einzelmilchproben aus allen milchliefernden Betrieben, wie das in Vorzugsmilchbeständen der Fall ist. Das wäre ohne weiteres möglich, wenn es sich dabei um einige 100 oder 1.000 Bestände handelte. Es geht aber beim Eutergesundheitsdienst des Tiergesundheitsamtes um etwa 660.000 Kühe in

nahezu 115.000 Beständen. Diese Bestände müssten alle wegen des verborgenen Charakters der Sekretionsstörungen häufig aufgesucht und die Milch der Kühe untersucht werden, da sie ja von heute auf morgen Sekretionsstörungen aufweisen können, die kürzere oder längere Zeit anhalten und auch wieder verschwinden können.
Müsste denn nicht ein allgemeiner tierärztlicher Eutergesundheitsdienst gerade daran scheitern, dass ein Tierarzt höchstens 3 Betriebe am Tage aufsuchen könnte, wenn er vorbildlich und einwandfrei arbeiten möchte; denn er braucht das steril entnommene Anfangsgemelk von jeder einzelnen Kuh für die Untersuchung auf Sekretionsstörungen und muss doch warten, bis die Kühe ausgemolken sind, um auch die Euter gut durchtasten zu können. Wie soll er aber in den Sommermonaten diese Untersuchungen durchführen?
Es stünden ihm also nur die Wintermonate mit der ohnehin sehr grossen Arbeitsüberlastung zur Verfügung. Mehr als 1 mal könnte er die Bestände in dieser Zeit nicht untersuchen.
Ähnlich ist es auch mit der Durchführung eines Eutergesundheitsdienstes, bei dem 2mal im Jahr — weil auch hier die Weidemonate ein häufigere Probeentnahme nicht zulassen — die Probenehmer der Milchkontrollvereine oder Molkereien in jedem Bestand die Proben entnehmen, und bei denen das Ergebnis in 90% der Bestände und mehr negativ lautet. Dabei stellt man sich immer wieder die Frage, ob eine zweimalige Untersuchung ausreicht, um auch wirklich sämtliche Sekretionsstörungen erfassen zu können und ob dieser starke Leerlauf in 90% der Bestände nicht doch vermieden werden kann.
Dieser letztere Gedanke hat uns schon seit 1951 bewegt. Gerade in dieser Zeit wurde die ABR-Probe nach Fleischhauer mit in den Eutergesundheitsdienst eingebaut und hat sich hierbei und später bei der Durchführung des Brucellöse-Bekämpfungsverfahrens vorbildlich bewährt. War es da verwunderlich, dass uns der Gedanke nach einem ähnlichen Verfahren zur Feststellung der Euterentzündungen kam? Immer wieder wurde dieser Gedanke von Karsten vorgetragen und hat uns auch unablässig beschäftigt, bis wir dann durch den Whiteside- und den California-Mastitis-Test zu Versuchen angeregt wurden, die aber scheitern mussten, da wir diese neue Untersuchungsmethoden anfangs falsch bewerteten.
Erst durch Dedié, Kielwein, Christ, Leidl und Schalm wurden wir erneut zu Versuchen angeregt.

Wir waren aber erst auf dem richtigen Wege, als wir die Dissertation von Herrn Kollegen Jaartsveld erhielten. Seine Brabanter Mastitis-Reaktion fanden wir geradzu ideal für unseren Eutergesundheitsdienst, da sie in so vorbildlicher Weise auch mit der Untersuchung der Milchproben auf Brucellose mit Hilfe der ABR gekoppelt ist. Zudem ermöglichen die konstruierten Apparate eine schnelle Entnahme und Untersuchung der Milchproben. Im Vergleich zu den bis jetzt von uns durchgeführten Eutergesundheitsdienstes wird die BMR auch kostenmässig am besten abschneiden.
In einem Grossversuch, der sich über ein halbes Jahr hinzog, hat Kollege Matschullat 12.000 Kannenmilchproben und Einzelmilchproben vergleichend untersucht.
Das Ergebnis der Kannenmilch-BMR zu der Einzelgemelkuntersuchung ist folgendes:

Beurteiling B.M.R.	Anzahl der Betriebe	Anzahl der Kühe	Einzelgemelksuntersuchung o.B.	Sekretionsstörung	Betriebe mit Mastitisproblem
negativ	240	2.000	96,0%	4,0%	—
fraglich	22	317	81,1%	18,9%	41%
positiv	23	449	69,5%	30,5%	87%

Abschliessend stellt er aufgrund seiner Untersuchungen mit der Kannenmilch-BMR fest:

1. Dieses zwar grobe doch in der Praxis für Massenuntersuchungen geeignet erscheinende Verfahren ermöglicht es, in kurzer Zeit auf einfache Weise einen Überblick über die Mastitishäufigkeit in einem grösseren Gebiet zu gewinnen.
2. Die Einfachkeit der Technik der BMR erlaubt ohne grösseren Aufwand kurzfristige Wiederholungsuntersuchungen, so dass man ständig einen Überblick über alle milchliefernden Betriebe hat.
   Gleichzeitig kann man durch Zwischenschaltung der ABR-Probe nach F l e i s c h h a u e r die heute üblichen Intervalle zwischen den Brucellose-untersuchungen wesentlich abkürzen.
3. Die BMR ermöglicht mit relativ grosser Sicherheit die Ermittlung besonders der Betriebe, in denen die Mastitis ein Herdenproblem darstellt.
   In einem Eutergesundheitsdienst, dem als Basis die Brabanter Mastitis-Reaktion gewissermassen als Sortiermethode zugrundeliegt, wird es somit möglich sein, den eigentlichen Mastitisbetrieben erhöhte Aufmerksamkeit zu schenken.
   Dort kann der zuständige Tierarzt mit Hilfe klinischer Euteruntersuchungen und bakteriologischer Prüfung von Viertel- und Einzelgemelksproben gezielt nach den Ursachen forschen und mit entsprechenden Massnahmen die Sanierung vorantreiben.

Wir sind daher der festen Überzeugung, dass wir mit Hilfe der BMR die Bestände mit einem Mastitisproblem mit allergrösster Sicherheit ermitteln können.

SAMENVATTING

In Duitsland worden reeds jaren bedrijven op mastitis onderzocht. De z.g. „Vorzugsmilchbetriebe", die rauwe melk voor consumptie mogen afleveren, worden elke maand door een inspecteur van de Veeartsenijkundige Dienst (Kreistierarzt) bezocht.

Naast klinisch onderzoek van de uiers worden koe-melkmonsters onderzocht. Dit betreft echter maar 50 bedrijven met 1000 runderen in een gebied met 115.000 veehouders.

Daarnaast is ook wel, op vrijwillige basis, door veehouders direct of via een zuivelfabriek aan een dergelijke mastitiscontrole deelgenomen. Halfjaarlijkse tot kwartaalsgewijze bezoeken met klinische controle en onderzoek van koemonsters geeft bij ± 10% een positieve bevinding.

De monsters worden op het laboratorium bacterioscopisch en zonodig ook bacteriologisch onderzocht.

Terwijl in het gebied van de Gezondheidsdienst Hannover deze bezoeken door een praktizerende dierenarts of een dierenarts van de Gezondheidsdienst worden gebracht, komt het in Duitsland ook voor dat hulpkrachten zijn ingeschakeld. Het klinisch onderzoek vervalt dan.

Afgezien van het feit dat deze methodiek duur is, wordt bij gebrek aan arbeidskrachten de uitvoering ervan zowel op het bedrijf als in het laboratorium onmogelijk. Dit zou nog sterker gelden indien het onderzoek algemeen zou worden.

In Hannover was men reeds in 1957 begonnen met een sedimentonderzoek van busmelkmonsters. Hoewel deze dienst zelf het koemonster-onderzoek prefereert, waren de zuivelfabrieken enthousiast over dit busmonsteronderzoek. Alleen de positieve bedrijven werden bezocht en nader onderzocht; de hoeveelheid werk verminderde sterk en het aantal positieve bevindingen nam daardoor sterk toe. Naast het sedimentonderzoek van busmonsters werd nadien ook de C.M.T. beproefd voor onderzoek van de busmonsters.

De laatste jaren werd de B.M.R. toegepast. Een vergelijking van de B.M.R. op busmonsters met onderzoekingen op de desbetreffende bedrijven gaf een goede overeenstemming.

Spreker meent te moeten stellen, dat:

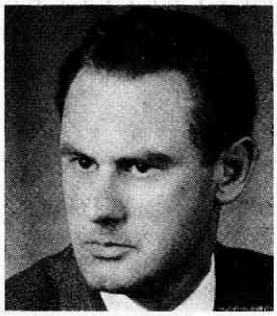
1. de B.M.R. een grove doch praktische methode voor massale onderzoekingen is; op een eenvoudige manier kan een overzicht, betreffende het voorkomen van mastitis bij runderen op de bedrijven in een bepaald gebied, verkregen worden.
2. de eenvoud en geringe kosten van het onderzoek een frequent busmonsteronderzoek rechtvaardigen.
   Het B.M.R. onderzoek kan, indien nodig, geschieden met melkmonsters waarop de A.B.R. reeds werd uitgevoerd.
3. men met de B.M.R., met relatief grote zekerheid de bedrijven die met een mastitisprobleem kampen, kan opsporen. Aan deze bedrijven kan men dan speciale aandacht gaan besteden.

Limits and possibilities of systematic mastitis control.

by O. RICHTER*)

Introduction.

Dr. Brus: *May I now introduce to you Dr. Richter from Münich, Germany.*
He is director of the Animal Health Service of that country and has the service of a modern laboratory where work is done in blood-group typing of cows and pigs.
He also has carried out investigations for some years in mastitis.
It's an honour for me to invite Dr. Richter to speak.



It is a well known fact that mastitis is a primary cause of cattle disease in all dairying countries. Every 3rd or 4th cow is assumed to have suffered from it one or another time of her life. The fact of mastitis being on the increase instead on the decrease makes it even more serious.
Eradication of tuberculosis and brucellosis and coital infections being finished in the herds, there was a demand for systematic mastitis control in Bavaria.

Only if the cause and the nature of a disease is known, a control can be planned. There are a series of mastitis definitions. The definition of mastitis being a wearing off disease of cows seems to me the best one.
Thus the most effective method for preventing this wearing off disease would be to make milk production cease altogether. This being impossible there is no other way but to look for any factors causing the disease and consequently eliminating them. This is the situation we shall have to face in mastitis control.
High milk production being the real cause of the disease cannot be changed. Our only possibility consists in establishing conditions under which the high productivity can be kept without any suffering of the organism or the udder.
This general explanation already marks the limits and possibilities of mastitis control.

Which are the factors favouring mastitis? There are especially:

bad milking;
bad hygienic and housing conditions;
influences of feeding;
biological influences (for instance simultaneous occurring infections and parasitic diseases, traumata or hormonal influences);
genetic factors and
bacteria.

In Bavaria we have established a mastitis control program since 1959. We think that this program should consist of:

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1. an efficient milk control service. This examination should make possible a perpetual and general supervision of the udder;
2. the employment of trained technicians being capable of diagnosing the predisposing factors and of preventing the causes;
3. a systematic and planned medicamental therapy with the aim of elimination of the mastitis germs out of the udder.

A current and general milk control is only possible with the help of a rapid and cheap information test. For this purpose we use the California Mastitis Test (C.M.T.) for years and test with this method the churn milk 3 or 4 times a year.

Up to date our experience shows that it is possible to detect mastitis herds by means of a churn-milk-test. Since one year we use in addition for a comparison the Brabant Mastitis Reaction (B.M.R.), developed by Dr. J a a r t s v e l d. We think to be able to detect more herds affected with mastitis by means of the C.M.T. than by means of B.M.R.

Of course, the churn-milk-test must be considered only as an information test. For a systematic mastitis control a cytological and bacteriological examination of milk-samples of each cow is necessary. This work is conducted by trained technicians, who collect milk-samples from each cow in herds suspicious of mastitis and deliver it at the laboratory of the animal health service.

We have tried to collect simultaneously informations on the housing conditions. This proved to be very useful, for systematic mastitis control should be initiated only in those herds, where the milking and housing conditions of the animals and the interest of the owner justifies the therapy. This is very important because we must be aware that every failure in therapy will affect the reputation of the udder health service.

Cows with severe pathological alterations of the udder don't pay for any therapy and should be culled.

For bacteriological examination we take a sample of each quarter of the udder under conditions as sterile as possible. Quarter samples have the following advantages as compared with milk-samples from all quarters mixed in one tube:

1. they allow a comparison among the udder quarters and a better distinction between physiological and pathological affects in the udder;
2. quarter milk examination enables us to treat each quarter separately;
3. a further advantage of examination milk from the single quarters is a rapid, simple and exact diagnosis, because there is no contamination by unspecific germs.

As soon as the result of cytological and bacteriological examination and the result of herd inspection is available, we are able to decide what to do further. According to the conditions encountered either veterinary therapy or an improvement of milking technique, feeding and housing is recommended. In most cases both is necessary. The succes of therapy is controlled at least twice by means of a single milk test. Animals resistant to therapy have to be culled.

It is also of great importance to give adequate and systematic information to the owners of herds on the influence of correct milking, feeding and

keeping conditions on the udder function by means of lectures and publications in agricultural journals.
It appeared that among all factors causing mastitis bad milking technic, especially hygienic and technical defects of milking machines take the first place. I am sorry to say that in my country though many milking machines are sold, there is no well organized customers service. If udder health service is to be successful we have to take care of this problem.
Veterinary therapy of the udder by means of antibiotics is only reasonable, when the predisposing factors can be abolished simultaneously. Mastitis eradication proved to be most successful with *Str. agalactiae* infections, provided the eradication program was continuously and thoroughly supervised. With staphylococcus infections a complete elimination of the germs was not obtainable, but in many herds a distinct decline in the incidence of the mastitis was obtained.

In my opinion with our present knowledge an eradication of mastitis is not to be expected. But if we are successful in curing herds and in controlling the total incidence of mastitis we shall be able to assume that the mastitis control program has been justified.

SAMENVATTING

In alle landen waar melkvee wordt gehouden, is mastitis een belangrijke ziekte. Men neemt aan dat 25 à 35% van alle koeien eens in hun leven mastitis hebben. Spreker vindt dit probleem zo belangrijk omdat bovengenoemd percentage toeneemt. In Beieren, waar tuberculose, abortus-Bang en de z.g. dekinfecties zijn uitgeroeid, vroeg de veehouderij om een systematische aanpak van het mastitisprobleem.
Om een ziekte te kunnen bestrijden is het noodzakelijk dat men de aetiologie van die ziekte kent. In dit kader zou mastitis een slijtageziekte genoemd kunnen worden. De meest „effectieve methode" zou dan zijn om met het melken te stoppen. Men zal de koeien moeten gaan houden op een manier waarbij het mogelijk is, hoge produktie met goede gezondheid te combineren.
De factoren die het ontstaan van mastitis bevorderen, zijn slecht melken, slechte hygiënische- en stalomstandigheden, biologische omstandigheden als trauma, hormonale invloeden, parasitaire en infectieuze aandoeningen en verder genetische factoren.
In Beieren is men in 1959 begonnen met opbouw van een programma ter bestrijding van mastitis. Hierbij denkt men aan: controle op de uiers en het melken, getrainde hulpkrachten die de predisponerende factoren kunnen onderkennen en dienaangaande adviezen kunnen geven, een sytematische medicamenteuze behandeling om de uierbacteriën te elimineren. De Gezondheidsdienst in Beieren gebruikt voor het massale melkonderzoek reeds jaren de C.M.T. en sinds een jaar daarnaast de B.M.R. Met de C.M.T. zijn volgens spreker meer positieve bedrijven te vinden dan met de B.M.R. Het massaal busmelkonderzoek kan alleen als eerste informatie dienen. Voor een systematische mastitisbestrijding acht spreker cytologisch en bacteriologisch onderzoek van kwartiermelkmonsters noodzakelijk. Bovengenoemde hulpkrachten zouden bij de monstername kunnen worden ingeschakeld.
Medicamenteuze behandeling acht spreker alleen verantwoord, indien de veehouder en de bedrijfsvoering aan bepaalde kwaliteiten voldoen, anders heeft de therapie geen resultaat en loopt de „uiergezondheidsdienst" gevaar haar naam te verliezen.
Koeien met ernstige afwijkingen dienen niet behandeld, doch geslacht te worden. Spreker geeft de voorkeur aan het onderzoek van kwartiermelkmonsters boven koemonsters.
Na het onderzoek van een bedrijf wordt beslist wat te doen. Veelal komt dit neer op verbetering van het melken en een medicamenteuze behandeling. Nadien vinden controles plaats om het resultaat te beoordelen.

Voorlichting via lezingen en publikaties vindt spreker zeer belangrijk. Naar zijn mening zijn slecht melken en defecten aan de melkmachines de voornaamste oorzaken van mastitis. In West-Duitsland is volgens spreker wel een goed verkoopapparaat voor melkmachines, maar geen goede service in de ruime zin van het woord.
Medicamenteuze behandeling heeft alleen nut als de predisponerende factoren ook weggenomen worden. De *Str. agalactiae* kan mogelijk uitgeroeid worden, de stafylokokken echter niet. Uitroeiing van mastitis achtte spreker niet mogelijk, wèl het terugbrengen van het aantal mastitis-gevallen tot een redelijk minimum.

Discussion,

following the lectures by Dr. Scheiner and Dr. Richter

Question: Prof. Van der Schaaf (The Netherlands):

I should like to ask Dr. Richter in what way he carried out the B.M.R.-test and how the Schalm-test?
Were these tests done at the same moment?
Was it the same milk?
Was there perhaps an old reagent?
Did you use sodium lauryl sulphate with an alkaline reaction of pH 12 or did you use sodium lauryl sulphate of pH 6?
I cannot understand how you found a difference between the results of both tests and I should like it very much that you explain the difference in these results.

Answer: Dr. Richter (Munich, Germany):

In our district we have the so called „Fleckvieh" and we have many troubles with staphylococci- and streptococci-mastitis. These two kinds of mastitis occur both in about 50 percent. The total cell-count of the staphylococci-mastitis in our „Fleckvieh" is very low in comparison with the other breeds of cattle.
Maybe the reaction of the udder to a staphylococci-infection is much lower than the reaction in other cattle. With staphylococci-mastitis the total cell-count according to Prescott and Breed is about 400.000 till 500.000 cells. This kind of mastitis we cannot find when we examine the can milk samples with the B.M.R. When we examine the quarter milk samples, we get good results with the B.M.R.
Your next question was how we compared the C.M.T. and the B.M.R. and I must say we have not done that at the same time. The difference in time was about 40 days. With the C.M.T. reaction we got about 10 to 12% positive herds by examination of can-samples and with the B.M.R. test we got about 5 to 6% positive herds.
Some time ago colleague Jaartsveld was in Munich and it was noticed that the pH was low, about 5 to 6. Afterwards we brought the pH to 12. With this reagent we examined 2 or 3 dairy factories, but we now found also more positive reactions with the Schalm-test than with the B.M.R.

Remark: Dr. Jaartsveld (Boxtel, The Netherlands).

It was remarkable that in Munich the results of the B.M.R. of quarter milk samples were very good, but the results of can samples were not right. It is important to get a good correlation between the total cell-counts of milk and the B.M.R. I cannot explain why the B.M.R. of the quarter milk samples gave good results and the B.M.R. of the can milk samples gave bad ones. And what about milk samples with a total cell-count of 400.000 per ml. out of which staphylococci are isolated?
Should one consider this milk as mastitismilk, or should one consider these staphylococci as contaminants?

Remark: Dr. Scheiner (Hanover, Germany):

I will emphasize the necessity to use in all German laboratories the same methods and the same reagent.

Question: Dr. Bratlie (Oslo, Norway):

During the demonstration at the farm we have seen the following interesting facts. We have seen a cow with a staphylococci mastitis, one quarter was atrophied.

The C.M.T. reaction was made from all the quarter milk samples. We have seen that the C.M.T.-reaction of the atrophied quarter was less than the reaction of the other quarters. The bad quarter with the chronic disease was less positive than the other three quarters, with more acute mastitis. Maybe from the atrophied quarter there are less leucocytes in the milk.

It would be very interesting for us to know, Dr. Jaartsveld, in which cases this method does not give good results.

Answer: Dr. Jaartsveld (The Netherlands):

Last Monday we did the B.M.R.-test of the 4 quarters of this cow and the B.M.R. test of all quarters was three or four points.

During the demonstration we should have done the C.M.T.-reaction and B.M.R.-test at the same time. In that way we could have tested the B.M.R. better. I agree with you that the C.M.T.-reaction of this atrophied quarter was less positive than the C.M.T.-reaction of the other quarters. It is possible that the total cell-count this morning at the moment we examined the atrophied quarter of the cow was less than some days ago.

Remark: Dr. Kielwein (Aulendorf, Germany):

We have had the following experience with staphylococci mastitis in cows in our district, in which we have „Fleckvieh" and „Braunvieh". After the tuberculosis-campaign we also have black-white and red-white cows.

We have compared a great number of results of milk-examinations. The „Fleckvieh" has about 0,4 percent *Streptococcus agalactiae*-infections. The black-white about 3,5 percent and the red-white cows have about 9 percent of *Streptococcus agalactiae*-infections. The cases of staphylococci-mastitis we have divided into two groups.

The first group are those milksamples out of which we have isolated *Staphylococci* and which milksamples have no more than 150.000 cells pro ml., the C.M.T.-reaction being negative.

The second group are those milksamples out of which we have isolated *Staphylococci,* the milksamples have high cell-counts and a positive C.M.T.-reaction.

The „Fleckvieh" cows, 11 animals, have a staphylococci-mastitis with a high cell-count, while 7 animals show a staphylococci-mastitis with a low cell-count.

In the red-white breed this figures are 8 : 1. I agree with Dr. Richter that the „Fleckvieh" has a less inflammatory reaction with a staphylococci-infection than the other breeds of cattle.

Dr. Bratlie said that the C.M.T.-reaction in the milksample of the atrophied quarter was less positive than the C.M.T.-reaction of the other quarters. I think it's quite normal, during the period the udder has mastitis, the cell-count will differ from time to time as well.

Question: Dr. Kraus (Germany):

In order to compare the mastitis reaction it should be desirable to make the method uniform in order to detect the same cell-count of the milk-samples. The question is, what test we have to use; Schalm-reaction, catalase-reaction, B.M.R.-test or some other kinds of reactions.

This problem is very important and therefore I ask this meeting: how to solve this problem?

Answer: Prof. D e d i é (Germany):

We have seen the very good standardized method to detect the total cells in the milk with the help of the B.M.R.-test at Boxtel. Professor K ä s t l i in Switserland used the modified Whiteside-reaction.
At Aulendorf we used the Schalm-test, but we take this reaction in tubes.
I think it would be more important that each of us continues, to see, what is the most suitable method for detecting abnormal milk.
I agree with Dr. K r a u s it would be excellent to use the same methods in all countries. I should prefer to wait one year or some more years. The time will come quick enough that we can judge the best method.

Remark: Dr. J a a r t s v e l d (The Netherlands):

In order to compare the different methods to detect bad milk I should like to tell you which method we use for this purpose.
About every two months about 35 milksamples are sent from one of the official laboratories to all laboratories of the Provincial Health Services, the University of Utrecht and the Central Veterinary Institute at Rotterdam. All laboratories perform the bacteriological examination, determine the total cell-count and perform the B.M.R.- test.
In this way it is possible to compare the results of the different methods from the different laboratories. Maybe it is also possible in Germany or other countries to do likewise. In this way you can proceed in standardizing laboratory methods.

Remark: Dr. R i c h t e r (Germany):

I think the problem is not very simple, because if we make comparisons between the different methods, we compare only the cell-count.
As I said before, in our country we have cases of clinical staphylococci mastitis in „Fleckvieh" with a lower content of cells than streptococci mastitis in for example the black-white cattle.
We have found clinical staphylococci mastitis with a cell-count of 500.000 till 600.000 pro m.l. If we compare these methods, we compare the cell-counts and that is **not** the problem.

Question: Prof. D e d i é (Germany):

I should like to ask Dr. R i c h t e r what he means by the word cell-count. Do you mean a total cell-count or the number of leucocytes pro millilitre of milk?

Answer: Dr. R i c h t e r (Germany): The leucocytes.

Remark: Prof. D e d i é (Germany):

So we should better use the term leucocyte-count, because I feel that much of the trouble, arising in diagnostic methods, comes from the misunderstanding of the terms: total cell-counts and leucocyte-counts. I should like to hear the opinion of this congress.

Question: Dr. Z w a n e n b u r g (The Hague, The Netherlands):

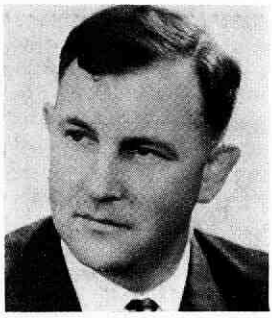
Mr. Chairman, I should like to ask colleague J a a r t s v e l d: what does he understand by the word cell-count?

Answer: Dr. J a a r t s v e l d (The Netherlands):

We have only worked with the total cell-count.
We have the opinion, that in many cases it is very difficult to differentiate between different kinds of cells. Therefore, for standardization of the B.M.R.-test we have used the total cell-count.

First results of a mass systematic mastitis test.

by D. H. J. BRUS*)



If one starts an eradication programme for a disease, you first need a method of diagnosis which is as simple, cheap and reliable as possible. In the case of mastitis this method must give the opportunity to divide herds into positive and negative ones. As regards the positive herds you have to investigate the individual animals, sometimes according to the same method of investigation, but mostly with other methods or combinations.
In the fight against Brucellosis in cattle the Abortus-Bang-Ringtest is such a method.
Periodical investigations of can-milk-samples can point out the positive herds. In these herds we use the serum agglutination and the Complement Fixation Test to detect the infected animals.
It must not be necessary to talk long about the borders between "negative, suspicious or positive" results of mass tests. All reactions near borders give too big a chance to make a false conclusion and to take a wrong decision. Conclusions may therefore only be drawn from real positive and real negative reactions.
If one declares a herd infected after one investigation, one has to be sure. If one likes to declare a herd free one can only do so after several periodical investigations. These investigations have to be done frequently. If there are suspicious reactions, decisions must be made in the following investigations.
Can we find such a simple method and can we use it as a „police-dog" in the fight against mastitis?
Cultural investigations of can- or tank-samples on the dairy-factories will give too many false positives, it will sometimes reach a 100% in tank-samples on the milk-delivery. Cultural investigations of cow- or quarter-samples will give purer results. But this sample-taking requires much work and labour is very expensive and in our country it will even become more expensive.
If we started a follow-up programme i.e. of cow- and quarter-samples cultural investigations every two months, it would be too expensive.
If you investigate once or twice a year, you miss a lot of positives. You will come much too late and it is still too expensive.
Bacterioscopic investigation of sediments of tank- or can-samples in the dairy-factories do not require so much work but yet it is too expensive to do it frequently.

We hope and think that the method of colleague J a a r t s v e l d which is the Brabantic Mastitis Reaction, will help us in mass diagnosing of mastitis, in dividing herds in groups with and without mastitis problems. In

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my opinion the value of this method is not so much in the sensitivity, but in the repeatability of this method. In the case of mastitis in my opinion we have to test it every month.

Four times a year we examine in our laboratory can-samples of every herd of our province, for testing on brucellosis with the help of the Abortus Bang Ring-reaction. Since January 1962 we made the B.M.R. on these same samples as well. Our farmers deliver about 100.000 cans daily. We investigate also more than half a million of milksamples with the B.M.R.

Table I.

Percentage of mastitis farms and percentage of cans with B.M.R. ••• or ••••

Period	% B.M.R. farms	Total of farms	% of cans B.M.R. ••• or ••••	Total of cans
1st. quarter '62	17,2	32.673	8.4	89.082
2nd „ „	15.9	32.731	5.9	100.253
3rd „ „	9.2	32.694	3.5	90.322
4th „ „	8.3	32.844	3.7	77.112
1st „ '63	11.4	32.570	4.7	84.332
2nd „ „	17.8	30.896	6.6	99.891
3rd „ „	11.9	31.552	5.7	92.336

Table II.

Percentage of mastitis farms and percentage of cans with B.M.R. ••• or •••• in the western part (Frisian cattle).

Period	% B.M.R. farms	Total of farms	% of cans B.M.R. •••• ••• or	Total of cans
1st quarter '62	21.8	9293	10.—	33131
2nd „ „	15.7	9423	5.1	39333
3rd „ „	8.1	6787	3.—	23923
4th „ „	8.6	9044	8.1	26931
1st „ '63	14.2	8592	5.1	33368

Percentage of mastitis farms and percentage of cans with B.M.R. ••• or •••• in the eastern part (Meuse-Rhine-Yssel cattle).

Period	% B.M.R. farms	Total of farms	% of cans B.M.R. ••• or ••••	Total of cans
1st quarter '62	14.7	17152	7.2	47884
2nd „ „	16.1	17228	6.4	61700
3rd „ „	9.6	15222	3.7	51654
4th „ „	8.2	16740	3.7	48313
1st „ '63	9.9	16430	4.3	49789

The first two questions we asked ourselves were:

1. are there seasonal differences in the percentage of herds with one or more positive can-samples?
2. are there local differences in this way?

The tables I and II show that there are more herds with positive reactions in spring and summer than in autumn and winter.

We see that these seasonal differences are more pronounced in the western part (Frisian cattle) than in the eastern part (Meuse-Rhine-Yssel cattle). We don't think that this is a difference in breed, but in the eastern part calving is more spread (1962: in the western part 25,4% of the first inseminations in July till December, in the eastern part 41,3%). May be you find more mastitis in cows in the first months of lactation. This can be the reason, but we have to do more investigations about this question.
There are also local differences as the maps show. These differences are not constant, but we find a trend in it. There are dairy-factories which are always black (as high as 20% herds with positive reactions) and there are some which are always white (less than 10%).

We can ask ourselves, whether it are always the same herds in which we find positive reactions?
We looked for them in three dairy-factories for the year 1962. We have taken three dairy-factories with successively a few, moderate and many positive reactions.

Dairy Factory	Asten	Riel	Raamsdonk
number of herds	308	118	196
always negative	62,9%	58,5%	40,3%
always positive	12,9%	24,1%	30,2%
varying	24,2%	17,4%	29,0%

I considered these questions with Mr. K e r k h o f and Mr. P e i j n e n b u r g, directors of the "Regionale Organen" which means organisations of dairy-factories, which look for the quality of the milk.
As you know, there is a difference in the price of good and bad milk; the difference is now about 10%, but this will grow in future.
The two gentlemen told me, „we see some agreement between your "mastitis-maps" and our "milk-quality-map" especially in the extremes".
Up till now, only in the western part of Holland are mastitis-tests incorporated with these payments orders (measures) and in this area the milk-quality investigations are:

1. methylene-blue reduction test,
2. sediment test,
3. organoleptic test.

For every test, the milk can get one to three points from good to bad. The final classification is the highest number of one of the three tests.

The methylene blue reduction test is done as follows: to 20 ml of milk is added 1 ml. of a methylene blue solution (1 tablet of 14 mgr. methylene blue chloride to 400 ml. of water). The tubes are placed in a waterbath of 37° C.
Interpretation: Decolorization within 1½ hour class 3, not decolorated in 3 hours class 1, dependent on the outside temperature.

Most of the bacteria present in an inflamed quarter don't influence this methylene blue test.
The sediment test is done to detect dirt in the milk. But a large amount of cells (leucocytes) can also give a positive test.
The organoleptic test of can-samples also will be influenced in very few cases only by mastitis-milk.

There will not be a direct correlation between the quality tests we use now in this country and the occurrence of mastitis at a farm. If we find a correlation between the quality of the milk delivered by farmers and the occurrence of mastitis in their herds, this correlation must be indirect. We hope to test this in the coming year for the whole province.
Up to now we have done this at four dairy-factories. These four factories have 1629 farmers.
In order to compare the milkquality and the occurrence of mastitis, we have given every herd a figure for milkquality and a figure for the B.M.R. test. For the milkquality there are 26 investigations of can-samples in a year. For this comparison we have taken the investigation in the days nearest to the day of B.M.R. testing. For the quality, the farmer gets every time a figure 1, 2 or 3. We have taken the average of the 4 investigations (also a farmer with successively 1, 2, 1 and 2 class of milk gets the figure $6 : 4 = 1{,}5$).
We have done the same for the mastitis (B.M.R. test) as follows: all can-samples with a negative B.M.R. get 1 point, one or more dubious ones get 2 points, one or more positive cantests get 3 points. The sum-total of points of the mastitis investigations divided by the number of investigations is called the Total Mastitis Investigation:

from 1 to 1,5 the Total Mastitis Investigation is called negative,
from 1,5 to 2 „ „ „ „ „ „ dubious,
2 and more „ „ „ „ „ „ positive.

For instance:

B.M.R.		Total Mastitis Investigation
— — — —	= 4 points : 4 = 1	negative
± — — —	= 5 points : 4 = 1,25	negative
± — ± —	= 6 points : 4 = 1,50	dubious
— ± — +	= 7 points : 4 = 1,75	dubious
+ — + —	= 8 points : 4 = 2	positive.

Table III shows that from 1629 herds there are 974 which are negative in B.M.R., 422 which are dubious and 233 which are positive. The percentage of first class milk decreases from 44,8 to 39,1 till 30,9.
The difference between the first- and second group is significant on a 5% level, the same holds good for the difference between the second and third group. When we look for the difference between the first and third group, it is highly significant (0,1% level).
The percentages of second class milk in all the three groups is almost the same. The third class milk increases from the negative to the positive mastitis group.
Here the difference between the negative and dubious group is significant on a 5% level and the difference between the dubious and positive group is highly significant (0,1% level), the same holds good for the difference between the negative and positive group.

Table III.

The B.M.R. test in comparison with the quality of the milk (test at 4 dairy-factories).

Negative B.M.R. (974 farms).

class 1	*class 2*	*class 3*
436 farms	311 farms	227 farms
44.8%	31.9%	23.3%

*) *) (***

Dubious B.M.R. (422 farms).

class 1	*class 2*	*class 3*
165 farms	136 farms	121 farms
39.1%	32.2%	28.7%

*) ***) ***)

Positive B.M.R. (233 farms).

class 1	*class 2*	*class 3*
72 farms	65 farms	96 farms
30.9%	27.8%	41.2%

x^2 — test

*) = significant 5 % level

**) = significant 1 % level

***) = significant 0.1 % level

If we take the three dairy factories one by one, we see the same line. Th'he differences then are not always significant, because the number of herds s is too small.

However we have to test this problem with much more material and I aram sure we will do this. I suppose that there are some circumstances at a farrm, which give a greater chance for occurrence of mastitis and delivering c of bad milk at the same time.

As you heard from Ir. C a z e m i e r, it is not easy to say what part c of handling and milking cows is most important here. But I will say, that it it seems that there is a correlation between the general management of th the farmer on one side and mastitis and quality of the milk on the other. Hefere the quality of the milk does not mean the cells in the milk or other direrect changes in the milk owing to the inflammation.

Therefore I think we have to fight against mastitis in combination wiwith the fight against bad quality of milk. We have also to cooperate with th the dairy-factories.

The main-point can't be the eradication of bacteria. Sometimes it will hehelp a special farmer or a special cow, but it will not bring down permanentntly the number of affected herds.

We have to bring and to hold the cows in a way that bacteria will n not have a great chance to cause inflammation.

Treatment of mastitis-cows will not bring down the number of positiitive herds and positive cows. We have to avoid mastitis.

At the end of my paper I will say it very black and white, I also exaggerarate on purpose:

a. Mastitis of cows is more a "disease" of the farmer, than a disease of cows.
b. The fight against mastitis should be incorperated in the fight for better milk-quality. Not only in an organized way (payment of milk) but also in a technical way.
c. Penicillin and other antibiotics don't give us a solution for the mastitis problem. They can only help to diminish the direct results of the inflammations. I will call it "Tranquillizer for the farmer".

SAMENVATTING

Om een ziekte massaal te kunnen bestrijden, moet men vooreerst over een diagnostiek beschikken, die zo betrouwbaar, eenvoudig en goedkoop mogelijk is.
De Abortus-Bang Ring-reactie — A.B.R. — was een reactie die aan bovengenoemde voorwaarden voldeed in het kader van de Abortus-bestrijding. Met behulp van deze methodiek is men in staat om door een onderzoek van busmelkmonsters bedrijven op te sporen die verdacht of positief zijn t.o.v. *Br. abortus.*
De runderen van deze bedrijven kunnen nader onderzocht worden met behulp van de serum-agglutinatie, complement bindingsreactie, A.B.R., enz. Bij elke massa-diagnostiek kunnen de uitslagen uitgesproken negatief, dubieus en uitgesproken positief uitvallen. Alle reactie-uitslagen die dubieus uitvallen, kunnen leiden tot foutieve conclusies. Het is daarom aan te raden in dergelijke gevallen het onderzoek te herhalen. Men moet zijn kracht niet zoeken in de verfijning van de reactie doch meer in de veelvuldige herhaling.
Het is juist een bedrijf na één positieve uitslag als „positief" aan te merken. Wil men echter een bedrijf „negatief" verklaren, dan moet het onderzoek vaker uitgevoerd zijn met bepaalde tussentijden. Voor de massa-diagnostiek op mastitis is het cultureel onderzoek van melkmonsters te omslachtig en te duur. Bovendien zijn er veel mastitisgevallen (± 35%), waarbij het bacteriologisch onderzoek negatief verloopt.
Wij menen dat de Brabantse Mastitis Reactie (B.M.R.), zoals deze door collega J a a r t s v e l d werd beschreven, als massaal diagnostisch onderzoek van mastitis bij runderen aan de bovenbeschreven voorwaarden voldoet.
Tenminste viermaal per jaar worden aan de Provinciale Gezondheidsdienst voor Dieren alle busmonsters die op de zuivelfabrieken geleverd worden (± 100.000) met behulp van de A.B.R. op brucellosis onderzocht. Sedert 1962 worden deze monsters ook met de B.M.R. op mastitis onderzocht. In totaal werden meer dan een half miljoen busmelkmonsters onderzocht met behulp van de B.M.R.
Uit dit onderzoek blijkt dat het aantal positieve B.M.R.'s in het voorjaar en de zomer hoger is dan in de herfst en de winter. Ook zijn er grote regionale verschillen. (zuivelfabrieken). Deze seizoensinvloeden zijn duidelijker waarneembaar in West-Brabant (zwart-bont vee) dan in Oost-Brabant (M.R.IJ.-vee). Waarschijnlijk speelt hierbij het feit een rol dat in West-Brabant het afkalven van de runderen meer in het voorjaar is geconcentreerd.
In het gebied van drie zuivelfabrieken nl. Asten, Riel en Raamsdonk met resp. weinig, matig en veel positieve B.M.R.'s werd nagegaan hoe de kwaliteitsbeoordeling van de melk, zoals deze nu wordt uitgevoerd, correleert met het voorkomen van mastitis op deze bedrijven.
De kwaliteitsbeoordeling heeft plaats op grond van: 1. reductaseproef; 2. wattenproef; 3. geur- en smaakproef.
De kwaliteitsbeoordeling wordt uitgedrukt in klassen, nl. 1e, 2e en 3e klasse, hier uitgedrukt in cijfers 1, 2 en 3.
De B.M.R. wordt uitgedrukt in punten. Aan een negatieve B.M.R. wordt het cijfer 1 toegekend, aan de dubieuze B.M.R. het cijfer 2 en aan de positieve B.M.R. het cijfer 3.

Op grond van deze cijferwaardering, zowel voor de kwaliteitsbepaling als voor de B.M.R. worden de kwaliteit van de melk van de drie bovengenoemde zuivelfabrieken en het voorkomen van mastitis op die bedrijven met elkaar vergeleken.
Over een bepaalde periode worden de gemiddelde cijfers, zowel wat betreft de kwaliteit van de melk, als de B.M.R. met elkaar vergeleken.
Het cijfer 1 — 1,50 — wordt bij beide negatief beschouwd,
Het cijfer 1,50 — 2 — wordt bij beide dubieus beschouwd,
Het cijfer 2 of meer wordt bij beide positief beschouwd.
Bezien we de drie zuivelfabrieken als geheel, dan is er in de eerste en derde groep een sterk significant verschil.
Dat wil zeggen dat op de bedrijven waar de kwaliteit van de melk goed is, significant minder mastitis voorkomt en omgekeerd. Het percentage van de eerste klas melk daalt resp. van 44,8 naar 39,1 tot 30,9 (zie tabel III) van de „mastitis" — via de dubieuze naar de vrije bedrijven.
De verschillen tussen deze grootheden van de 1e en 2e groep of de 2e en 3e groep zijn eveneens significant (5% level). Deze significantie gaat niet voor elke zuivelfabriek apart geheel op, omdat hier de aantallen te klein zijn.
Zoals Ir. C a z e m i e r naar voren bracht, is het moeilijk vast te stellen welk punt betreffende de bedrijfsvoering of welk punt bij het machinaal melken extra belangrijk is voor het ontstaan van mastitis.
Er bestaat volgens de spreker een correlatie tussen de algehele bedrijfsvoering van een veehouder enerzijds en het voorkomen van mastitis bij de runderen en het resultaat van de kwaliteitsbepaling van melk anderzijds. Vandaar dat het logisch is de mastitis-bestrijding samen op te bouwen met die instanties, die zich bewegen op het terrein van de kwaliteitsbepaling van de melk en de daaraan gekoppelde uitbetaling.
Omdat het onmogelijk is de mastitis-verwekkende of mastitis-onderhoudende bacteriën uit te roeien, moet het doel van een mastitis-bestrijding voornamelijk gericht zijn op het zo hoog mogelijk houden van de weerstand van de uier. Antibiotica als zodanig vormen over het geheel genomen geen oplossing voor het mastitis probleem.
Tenslotte stipuleert de schrijver het heel duidelijk als volgt:

1. Mastitis bij runderen is in de eerste instantie meer „een ziekte" van de veehouder dan van de runderen.
2. Zowel organisatorisch als technisch moet de mastitis-bestrijding worden opgezet samen met de instanties die zich bezig houden met de kwaliteitsbepaling van de melk.
3. Antibiotica vormen als zodanig geen oplossing voor het mastitisprobleem. Het zijn hierbij meer verdovingsmiddelen voor de veehouders, dan middelen om de mastitis terug te dringen.

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General discussions

on September 25th, 1963, at the end of the second day of the Congress.

Remark: Dr. K l a s t r u p (Denmark):

If you make the bacteriological examination of the can-samples and at the same time you make a bacteriological examination of the quarter-milk-samples you can get a correlation of about 100%. So we feel in Scandinavia — I don't know if I can speak on behalf of my colleagues from Norway and Sweden — that the bacteriological examinations are reliable in the control of mastitis.

An other point concerning the variation of the B.M.R. in the different

seasons of the year, I don't know how the calving season is in your country. In one dairy-district of about 220 herds, every week we made a total cell-count, at the end of the year we compared the result with the calving season and there was a correlation between the level of the total cell-count and the calving season.

Answer: Dr. B r u s (Boxtel, The Netherlands):

I should like to put forward that I also agree with you that the bacteriological examination can be useful in controlling mastitis, but first I must have a good and cheap method by means of which you can find out the herds and the cows which have problems with mastitis. And when I have the infected quarter, then I like to take a bacteriological examination.

If you take the bacteriological way to detect the farm and the cows, it costs a lot of money and a lot of labour. Labour is already very expensive and is becoming more expensive. It is already very difficult to get people, who take milk-samples in controlling the fat-content of the milk. And therefore, when we want to start an organized campaign against mastitis by taking samples from the quarters for bacteriological examination, it is impossible for us to do so. There is a proverb in the Dutch language that says „The calf will be bigger than the cow". Do you understand that? It is not to be paid, it is too expensive.

And as to the other matter, I told you there is a difference of B.M.R.-test during the different seasons of the year. The difference between the seasons is more pronounced in the western-part of Brabant than in the eastern-part.

In the western-part calving more or less takes place in the beginning of the year. It is possible, but I am not quite sure, you have the greatest frequency of mastitis in cows which have calved about five months ago. We have to prove it.

Remark: Prof. S t e g e n g a (Wageningen, The Netherlands):

It will be dangerous to conclude from these investigations that there will be a narrow correlation between mastitis and the hygiene of the milk. It can be a coincidence without a real relation.

For example: your percentage of positive herds is very closely related with the number of cows in the herds. If you have a herd of 20 cows, the chance of having a cow with mastitis is at least twice as great as in case of a herd of ten cows.

The same holds good for the quality of the milk. With bigger herds you have more people to handle milk than in smaller herds. On bigger farms the quality of milk can go down without trouble of mastitis, so in fact there seems to be a relation, but I do not think that there is a *real* correlation.

Answer: Dr. B r u s (The Netherlands):

I agree with you, you have a greater possibility to find one cow suffering from mastitis on bigger farms than on smaller farms. But as far as we know there is no correlation between the number of cows and the quality of the milk delivered and the number of cells pro millilitre in this milk.

Remark: Prof. S t e g e n g a (The Netherlands):

It must be so!

Answer: Dr. B r u s (The Netherlands):

I am not the same opinion. If you compare herds of ten cows to herds of 20 cows you don't find more than twice the number of cases of mastitis. My conclusion is not that mastitis-milk always will be classified as of

third class quality. If you have a farmer, who manages his herd in a specially bad way, you have more chance to get mastitis and to get third class milk.
These two phenomenons: mastitis and a bad quality of milk have the same cause, namely bad management.

Remark: Drs. M o l (Amsterdam, The Netherlands):

Mr. Chairman, it will be good to prevent some misunderstanding.
Dr. B r u s wants to state that farmers having poor management practices also produce unclean milk. That is what Dr. B r u s means.
There should be a relation between the hygienic quality, determined by the sediment-test, methylen-bleu-test and the smell of the milk and of the incidence of mastitis in cows. Poor practices of management of the cows coincide with a bad quality of the milk and bovine mastitis.

Question: Dr. E d w a r d s (England):

Dr. B r u s, I would like to ask you some questions.
You said that the penicillin is a tranquillizer for the farmer. That is a very interesting remark. I cannot agree with you as regards that statement. Penicillin will not remove mastitis, but we do know certain forms of mastitis which can be eradicated by using penicillin, if applied properly. For example the mastitis caused by *Streptococcus agalactiae*. I don't think that you can generalize that penicillin is a tranquillizer.

Answer: Dr. B r u s (The Netherlands):

I told in the beginning that I would tell you things very black and white. I also exaggerate.
I agree with you that good results may be obtained with penicillin in a herd with *Str. agalactiae,* in that you can cure some cows and also help that farmer for a while. But when you like to help your country, to decrease the number of problem herds, penicillin will not be the solution. You know what L i v o n i found, when the *Streptococcus agalactiae* was eradicated, another streptococci was coming. And therefore when we speak about an organized fight against mastitis — not only against *agalactiae*-mastitis — you do not come to the end with penicillin. If we find a farmer, who is in trouble, we have to help him. But when I speak about an organized fight against mastitis, I am thinking of preventing. And when I come at a farm with a lot of mastitis,I come too late in the sense of an organized mastitis control.
We also have to investigate what is the best way of management to prevent mastitis on these farms.

Question: Dr. K r a u s (Hanover, Germany):

In how many cases of mastitis did you fail to have good results and what have you done in such cases?

Answer: Dr. B r u s (The Netherlands):

I only told you about the results of our mass investigation about mastitis diagnosing and the correlation between mastitis and quality of the milk. Therefore I can't answer your question.

Remark: Dr. K l a s t r u p (Denmark):

I agree with Dr. B r u s that the prevention of mastitis is very important and I think all of us can subscribe that.
But another question is this: Dr. B r u s said that L i v o n i has found that when you eradicate *Str. agalactiae,* other infections come in. In my opinion L i v i o n i didn't prove that.

If you have a herd, infected with *Str. agalactiae* and no other infection can be seen, no other infection comes in when you eradicate *Streptococcus agalactiae*. That is why we in Scandinavia prefer to eradicate this specific diseases of *Streptococcus agalactiae*. But at the same time we carry out cell-count or C.M.T. in order to find herds with mastitis problems caused by other reasons.

Answer: Dr. Brus (The Netherlands):

I thank you very much. So you have in Scandinavia herds with mastitis problems, in which you only find *Streptococcus agalactiae*. We seldom find only one bacterium in our problem herds.

Remark: Dr. Jaartsveld (The Netherlands):

We have the impression that in many of our herds different micro-organisms play a part in causing mastitis. From one quarter one isolates *Str. agalactiae*, out of the other *Str. dysgalactiae, Str. uberis,* staphylococci and so on.

Therefore we have the impression that mastitis is primarily not an infection-disease. Mastitis is caused by a general lowering of resistance of the udder. And when the udder has a low resistance, that bacterium comes in which is nearest to the udder.

Remark: Dr. Brus (The Netherlands):

I think you have two starting points in combating mastitis;
Firstly: by bacteriological examination of the can-samples.
Secondly: by estimation of the cell-count of the can-samples.
You can have a starting point of finding bacteria in can-samples and then you find many herds with streptococci. We have found the same thing, but when you come at the farms where *Streptococcus agalactiae* is present, you don't always find a herd with a mastitis problem.
If you face mastitis in that way, you use can-samples for bacteriological examination and if you have a good penicillin regulation, you get results on these farms and you have a lowering of the cell-counts.
Next to this you have the other farms and I think that is the other point of view. When you start the mastitis- campaign to find out the problem-herds by estimating the total cell-counts of the can-samples, you can start with these problem-herds, because you cannot go to all the farms. It is impossible to do so. You have to go to the farms on which they struggle with the problems.
In some herds *Streptococcus agalactiae* is very infectious and in other ones *Streptococcus agalactiae* is not infectious.
If we will face the mastitis in all cases for an esthetic aim as well we try to get a low cell-count in the milk of all farms.

Remark: Mr. Kingwill (England):

The efficiency of antibiotics and of penicillin especially, is very different on streptococci and against staphylococci. I think we will all agree in this. I think in England it is the opinion that since the eradication of *Streptococcus agalactiae*-infections in many herds the incidence of three quarter cows is less common. In the last years'work we have observed that in many cases the serious clinical cases of mastitis have been caused by streptococci namely *Streptococcus agalactiae-, dysgalactiae-* or *uberis.*
Although the staphylococci infection is a great problem, and in some herds it is the most common pathogen to be found, it is relatively not so important in clinical disease. In our experimental work the new infections with staphylococci remained high. It is more encouraging that the streptococci infection can be reduced to a greater extent.

Remark: Dr. F u n k e (Örebro, Sweden):

In Middle-Sweden we have a higher occurrence of mastitis in small herds than in big herds. Maybe the reason is, that in big herds they have more young cows than in small herds. In middle Sweden we have very few infections with *Streptococcus agalactiae*. *Streptococcus agalactiae* is no problem in Middle Sweden. Maybe this is another reason why we have more infections in small herds. We possess a health control that agrees with the method Dr. R i c h t e r reported to a large extent.

Remark: Prof. S c h a l m (U.S.A.):

I should like to make some remarks about the difference in pathogenicity of *Streptococcus agalactiae* and *Staphylococci.*

In 1930 S t a b l e f o r t h, E d w a r d s and M i n e t t, were perhaps the first people to show that *Streptococcus agalactiae* can be eradicated. They were also the first to show that one can live with *Streptococcus agalactiae* in case of good management.

Unfortunately the moment comes in the bovine lactation cycle, that she must be dried off and you can't milk her completely and regularly. And it is my observation that if *Streptococcus agalactiae* will be left in the udder of the cow during the dry-period, more scar tissue will penetrate in the udder.

With staphylococci, I believe there is less scar tissue and personally, if I were a farmer and I know about mastitis what I know now, I should elect stapylococci instead of *Streptococcus agalactiae* in my herd, during the dry-period, and if possible I should do my utmost best to eradicate *Streptococcus agalactiae.*

Question: Dr. K l a s t r u p (Denmark):

I should like to put to Dr. R i c h t e r the following question.

He said that after some bacteria were isolated out of cases of mastitis he checks these cases after three weeks and later on after three months. I should like to ask him: what results did you have by this checking?

Answer: Dr. R i c h t e r (Germany):

It is not possible for me to answer this question exactly. I agree with Dr. K i n g w i l l that if it is possible to eradicate the chronic mastitis cases and to use penicillin with the non-chronic cases, we may have a good result in about 85%.

With staphylococci mastitis we have very bad results with antibiotics too.

Question: Prof. V a n d e r S c h a a f (The Netherlands):

There is one question I didn't hear up till now.

Are there some figures about decrease of the production of the cow in kilograms of milk, when there is an infection by *Streptococcus agalactiae* or a staphylococci infection?

I think we have two groups of mastitis; there is one group caused by *Streptococcus agalactiae* and one group caused by the other udder streptococci and staphylococci.

Is there anybody who can tell something about the diminishing production in case of mastitis?

Answer: Dr. K l a s t r u p (Denmark):

I can tell about the results of W i l s o n about 1947. He found a lower yield of milk of the cows infected with *Streptococcus agalactiae* of 10 till 15% and in addition I can mention the examinations of Dr. L i v o n i. He compared the successive lactations of a group of cows suffering from *S.agalactiae*-infections to those of a group of cows which were not infected

with this germ. He found that the diminishing of milkproduction of the *S. agalactiae* was about 8%.

During this discussion the following statement of Dr. Wisniowski was read out:

The Statement of the Lecturer Dr. George Wisniowski (Poland) during the Congress at Boxtel, North Brabant, The Netherlands) in September 1963.

Gentlemen,

Feeling honoured by your invitation to the Mastitis Congress I must apologize for my absence caused — to my regret — by having got the invitation too late. I want to emphasize that I am fully aware of the importance of such a meeting of the researchers interested in the problems of mastitis. I hope that such meetings will take place in future, too.

Being the only representative of my country I wish to assure you that — as Poland is the fifth of the producers of milk in the world — (12,5 milliard litres yearly) we are greatly interested in the progress of science and in the organization of mastitis control in cows. Our economy and agriculture fully appreciate the negative influence of mastitis upon the efficiency of milk in cows. The result of this opinion is the concentration of the investigations of mastitis in the Department of Animal Hygiene of the State Veterinary Institute. In our Department of Animal Hygiene we make immunological investigations planned for a long time, and besides this, since two years, we make observations as to the usefulness of two screening tests in mass diagnosis such as the Hotis test and the cytological test according Schalm. Both these tests seem to be very valuable in comparative investigations (based on the classical bacteriological test and on the cytological analysis and cell count designation). Here I want to say, that we met with the kind understanding of Professor Schalm at the adaptation of the Californian Mastitis Test and got concrete help from our colleague Doctor Jaartsveld whom I want to say here my best thanks. It is our opinion that — at least in the conditions of our country — the introductory mass diagnosis should be based upon both of the mentioned tests. They supplement very well, one describing the ethiology of infections for orientation, the other one describing the irritation of udder, and it is of greatest importance that they may be applied by practical veterinarians in the field (terrain). The results of the investigation of individual cows of great herds when both the tests were applied, allow to make a rather detailed appreciation of the herd and to get a suggestion as to the necessity of the elimination, the treatment and the sanitary plan of milking. This may be done by the owner, whereas the detailed investigations in particularly justified cases made with classical methods would be — such is our opinion — the task of the specialistic laboratories.

However, as we have not such a large experience as, for instance, our Hosts, therefore this Congress is of a still greater value for us. I am sure, that it is a rather actual and necessary meeting which ought to initiate further congresses of this kind.

I also beg to notice, that in case of the standardization of methods and the working out of some organizing scheme for the uniformity of mastitis control, valuable comparable results could be got in the particular countries taking part at this Congress, and those results could be the subject of further mutual discussions.

At the end I want to thank my Colleagues and Hosts for giving me the possibility to take the word at least in this way.

Demonstrations

The organization and execution of milk-sample taking.

by M. GALEMA*)

The organization.

Every three months milk-samples have to be taken from 64 dairy-factories with a total of 70-90.000 cans. Therefore the province "Noord-Brabant" is divided into several sections each of 3-5 dairy factories and in each section a man is employed to take the samples.
The laboratory at Boxtel has a capacity for the examination of 10-15.000 samples in the afternoon, so execution of this test takes 9 days. The delivery and collection of the sampling outfit and the samples is carried out by car.

Sampling outfit.

1. Stainless steel sample-taker of one millilitre.
2. Perspex screen plate, prevents spilled milk from coming into other tubes.
3. Perspex tube-containers: for a hundred milk-tubes. Every container has a serial number. The places for milk-tubes are numbered from 1 to 100.
4. Milklist: A list in duplicate. The numbers of the milk-cans and the serial number of the tube-container are noted on this list, so it is possible to trace the cans back to their origin.
5. Milkchest: There are two kinds of chests; one contains 600 tubes, the other one 1000 tubes. They serve for the transport of the samples. Two foamrubber pads cover the tubes in the containers.

Sample taking.

The sample-taker is dipped in open position into the milkcan and is taken out of the can in closed position after thoroughly stirring, whereafter the sample is transferred into one of the milk-tubes. The sample-taker is not cleaned after every sample taking; the few drops of milk left in the sample-taker are rinsed by stirring in the following can. As a result of the great dilution, this milk does not influence the reaction.
After every 20 samples the screenplate covers the tubes filled with milk and so prevents spilled milk from coming into other tubes.
The numbers of the milkcans and the serial-number of the container are noted on the milklist.
The containers and milklists are placed into the chests, ready for transport to the laboratory.

SAMENVATTING.

Organisatie

Tenminste eenmaal per kwartaal moeten 70—90.000 melkmonsters genomen worden op 64 zuivelfabrieken. Om dit te organiseren is Noord-Brabant in 2 delen verdeeld.

*) Mr. M. Galema; Animal Health Service of North-Brabant; Rechterstraat 80, Boxtel, The Netherlands.

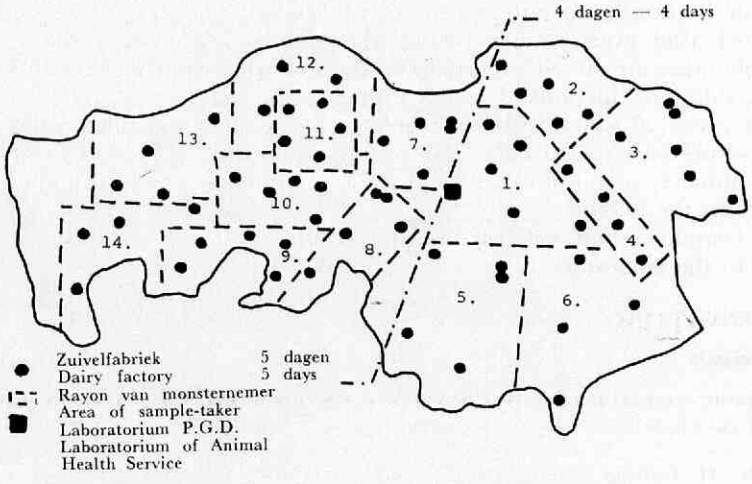
Een deel omvat alle zuivelfabrieken ten Oosten van Boxtel, het andere deel de fabrieken ten Westen van Boxtel. Ieder deel is onderverdeeld in rayons van 4—5 zuivelfabrieken, waarbij voor elk een monsternemer is aangetrokken.
Door op een onderzoekdag uit ieder rayon van resp. Oost- of West-Brabant een zuivelfabriek te bemonsteren kunnen in 4 dagen alle zuivelfabrieken in het Oostelijk deel en in 5 dagen alle fabrieken in het Westelijk deel van Brabant bemonsterd worden. Per dag worden zo 10—15.000 monsters genomen en op het laboratorium onderzocht. Het bezorgen en ophalen van de vóór het monsternemen benodigde materialen wordt door de Gezondheidsdienst verzorgd.

Uitrusting

1. Melkmonsterlepel: van roestvrij-staal met een inhoud van 1 ml.
2. Plastic schermkap: wordt na iedere 20 monsters over de genomen monsters geschoven en voorkomt, dat melk gemorst wordt in de andere buisjes.
3. Melkrek: Een uit kunststof vervaardigd rek met plaats voor 100 melkbuisjes. Ieder rek heeft een reknummer. De plaatsen van de 100 buisjes zijn genummerd van 1 t/m 100.
4. Melklijst: Een lijst in duplo waarop de busnummers van de genomen monsters genoteerd worden. De no's 1 t/m 100 corresponderen met de nummers van de melkbuisje. Het genoteerde reknummer komt overeen met het rekno. waarin de monsters geplaatst zijn.
5. Melkkist: Een houten kist met plaats voor 6 of 10 melkrekken. Een met plastic folie overtrokken plaat schuimplastic voorkomt, dat tijdens het transport melk gemorst wordt.

Monstername

Uit iedere aangevoerde bus wordt een melkmonster van ± 1 ml. genomen. De melkmonsterlepel wordt geopend in de melkbus gestoken en na enkele malen goed roeren er gesloten uitgehaald. Op deze manier is het niet nodig, dat de lepel na ieder monster wordt schoongemaakt. Praktijkproeven hebben uitgewezen, dat door de grote verdunning van de achtergebleven melk bij A.B.R. en B.M.R. geen invloed wordt uitgeoefend op de uitslag van de reactie. Nadat de monsters genomen zijn, worden de rekken in de kisten geplaatst en zijn dan klaar voor transport naar het Laboratorium te Boxtel.



Explanation about laboratory technics for mastitis-examination.

by E. VAN WERVEN*)

History of the Brabant Mastitis Reaction (the B.M.R.).

When we started with the investigations of mastitis, the Whiteside-reaction was used. 3 Drops of milk were transferred on a glassplate and mixed with 1 drop of NaOH 1 n (4%).
This method was followed by a plate-reaction between milk and a surface-active agent, Na-T-pol (Shell). Therefore 3 drops of milk were mixed with 3 drops of a 10% T-pol solution, with the aid of a stirring rod.
A very great number of milk-samples, which we received in plastic tubes at our laboratory, had to be examined. The plate-method was modified into the tube-method. The California Mastitis Tube Test (C.M.T.T.) was born.
The amount of inflammatory cells in the milksamples was also counted. It seemed that there was a very good correlation between the number of inflammatory cells in the milk and the viscosity, which arised by mixing in a tube 0,6 ml. of milk and 0,4 ml. of a 10% Na-T-pol solution, with the aid of a stirring rod. Later on a 2% sodiumlaurylsulfate solution was used.
When a dilution of the milksamples was made with negative milk, an impression was obtained regarding the amount of inflammatory cells, which was present in the milk.
In order to estimate the number of cells without dilution, the viscosity of the mixture of milk and Na-T-pol can be determined using a glass funnel with a capillary of 20 mm. length and a diameter of 1,3 mm.
In this way the Brabant Mastitis Reaction (B.M.R.) is performed.

Demonstration of the B.M.R.

The milksamples, used for the investigations on mastitis, are first used for the brucellosis investigation by applicating the Abortus Bang Ring-reaction (A.B.R.).
Therefore to each tube containing 1,0 ml. milk is added 1 drop of A.B.R.-antigen. The samples are shaken well, placed in an incubator and after 1 hour they are judged. A positive A.B.R. reaction shows a blue coloured cream layer and decoloured milk, and a negative reaction shows blue coloured milk and a white cream layer.

After this, the samples are used again for the investigations as to mastitis. As most cells are to be found in the cream layer, the samples are shaken well to mix these cells homogeneously through the milk.
We use 0.6 ml. of this milk and therefore the supernatant layer is removed by means of a sucking apparatus. In order to prevent inflammatory cells from remaining on the needles of the sucking apparatus, the latter are rinsed with running water, every time after use.

*) Mr. E. van Werven; Animal Health Service of North-Brabant; Rechterstraat 80, Boxtel, The Netherlands.

To each tube is added 0,4 ml. of a 2% Sodium Laurylsulfate solution. The pH of this solution is corrected by NaOH to pH 12. In this way we can examine the milksamples after a longer time (± 48 hours).
The samples are shaken well. By the presence of inflammatory cells the nucleus breaks open and the desoxyrubosenucleic acid (D.N.A.) comes free.
This D.N.A. lends a rise in viscosity to the samples. This viscosity is according to the amount of inflammatory cells, which is present in the milk. The grade of viscosity is determined by means of the time of flowing through.
For this, the samples are put in capillaries. This takes place by means of tumbling 100 samples at a time in 100 corresponding capillary tubes. These tubes are blocked by a block-plate. Now the time of flow through is determined. As soon as the tubes are lifted from the block-plate, the time of flow through starts, and after 5 seconds a picture is taken. This is repeated after 10, 20 and 60 seconds. Samples, which have flown through the capillaries within 5 seconds have a negative B.M.R. The amount of cells is not greater then 200.000 per ml.
Samples which are in the tubes after 5 seconds get 1 dot; it is the milk which contains about 400.000 cells per ml. Samples which are still in the tubes after 10 seconds get 2 dots, corresponding with about 800.000 cells per ml. 3 dots are noted for those samples, which contain about 2.000.000 cells per ml. Samples, which are in the tubes after 60 seconds, are marked with 4 dots. The amount of inflammatory cells is about 5.000.000 per ml.
When time is up the sets with capillary tubes are removed quickly to make place for the following sets. In this way it is possible to investigate about 10.000 samples an hour with the aid of 6 laboratory assistants.
The following day, the results are noted on the corresponding lists.

SAMENVATTING.

In een overzicht wordt de ontwikkeling van de B.M.R. gegeven. Via de Whiteside reactie (melk + loog), de C.M.T. (reactie tussen melk en T-pol op een glasplaat), de C.M.T.T. (melk + T-pol in een buisje) is de huidige B.M.R. ontwikkeld.
Deze reactie maakt het mogelijk een groot aantal melkmonsters op snelle en goedkope wijze te onderzoeken op aanwezigheid van ontstekingscellen.
Eerst worden de melkmonsters voor het abortus-onderzoek gebruikt. Dit geschiedt met behulp van de Abortus Bang Ringreactie (A.B.R.)
Direct nadat deze reactie is afgelezen, wordt met de B.M.R. begonnen. Deze directe opeenvolging is gunstig voor het verloop van de B.M.R., gezien de optimum temperatuur bij 20 à 25° C.
Daartoe wordt het volume van ieder buisje tot 0,6 ml teruggebracht en hieraan 0,4 ml T-pol oplossing toegevoegd. Bij aanwezigheid van ontstekingscellen worden door een reactie tussen deze cellen en het toegevoegde reagens de ontstekingscellen stukgemaakt, waarbij een slijmige kernvloeistof (D.N.A.) vrijkomt.
Naarmate het aantal ontstekingscellen groter is, neemt de slijmigheid toe. Deze slijmigheid wordt met behulp van nauwe doorstroomcapillairen gemeten.
De uitslagen worden fotografisch vastgelegd.

Mass detection of antibiotics in milk.

by F. H. J. JAARTSVELD*).

In order to test many milksamples for the presence of antibiotics, we have taken the view that the tube-containers form the basis of the method. Therefore rectangular moulds are made, with the same surface as the tube-container.
About 160 ml. 2% agar medium is mixed with ± 25 ml. *Sarcina lutea*-culture and poured out in this mould.
After that a punching-apparatus with 100 legs (photo 1) is placed on the bottom of this mould. When the agar is coagulated the punching-apparatus is removed from the filled mould and 100 little holes are left.
With an antibiotic-drop-apparatus (photo 2), three drops of milk are removed out of every tube of the tube-container (photo 3) and transferred in the corresponding hole of the mould (photo's 4 and 5).
The mould is incubated at 37° C for about 20 hours.
All milksamples examined for brucellosis antibodies with the Abortus-Bang-Ring-reaction are as well suited for the method of penicillin detection as the fresh milksamples are.
In this way it is possible to examine the milksamples as to brucellosis, as to the presence of antibiotics and of inflammation cells with the aid of the B.M.R. and in a simple, easy and cheap way.

SAMENVATTING.

Hierbij wordt uitgegaan van de bestaande melkrekken, die worden gebruikt voor het A.B.R.- en B.M.R.-onderzoek.
De antibiotica in de melk worden aangetoond in rechthoekige platen, welke dezelfde oppervlakte hebben als de bovengenoemde melkrekken. Deze platen worden gegoten met ± 160 ml 2% agar en ± 25 ml *Sarcina lutea*-cultuur. Daarna wordt in de vloeibare agar een ponsapparaat geplaatst met 100 pootjes die onderling dezelfde afstand hebben als de buisjes in het melkrek.
Als de agar gestold is, wordt het ponsapparaat verwijderd zodat 100 gaatjes in de agar overblijven.
Met behulp van een antibioticum-druppel apparaat wordt in één keer vanuit de 100 buisjes staande in het melkrek 3 druppels melk opgezogen en overgebracht in de overeenkomende holten van de agar-plaat. Deze agar-plaat wordt ± 20 uur bij 37° C bebroed.
De melkmonsters die een remmingszône vertonen kunnen dan direct onderkend worden. Melkmonsters die meer dan 0,02 E penicilline per ml bevatten, kunnen met deze methode worden aangetoond.
Op deze wijze is het mogelijk alle kwartaal-busmelkmonsters — elk van 1 ml melk — komende van de zuivelfabrieken te onderzoeken met behulp van de A.B.R. op abortus Bang, met behulp van de bovenbeschreven methode op het voorkomen van antibiotica en met behulp van de B.M.R. op het voorkomen van ontstekingscellen in de melk.

*) F. H. J. Jaartsveld D.V.Sc.; veterinary surgeon to the Animal Health Service of North-Brabant; Boxtel, Rechterstraat 80, The Netherlands.

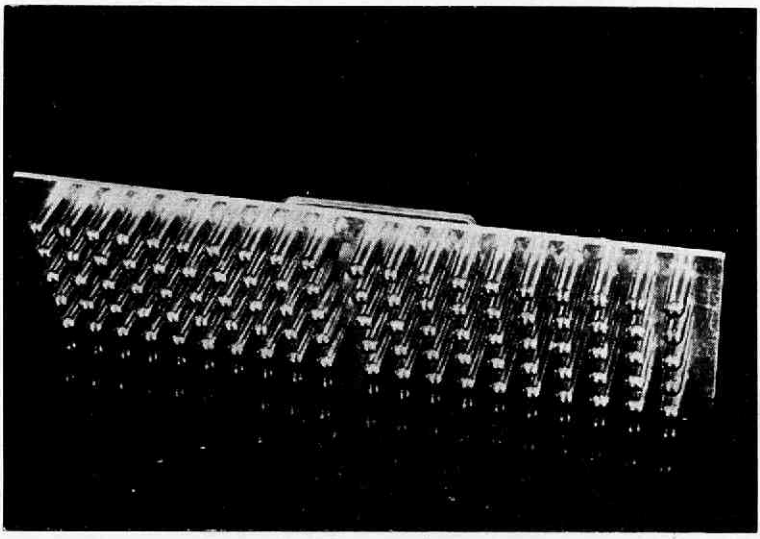


Photo 1. The punching-apparatus.

(photo M. W. P. Galema).

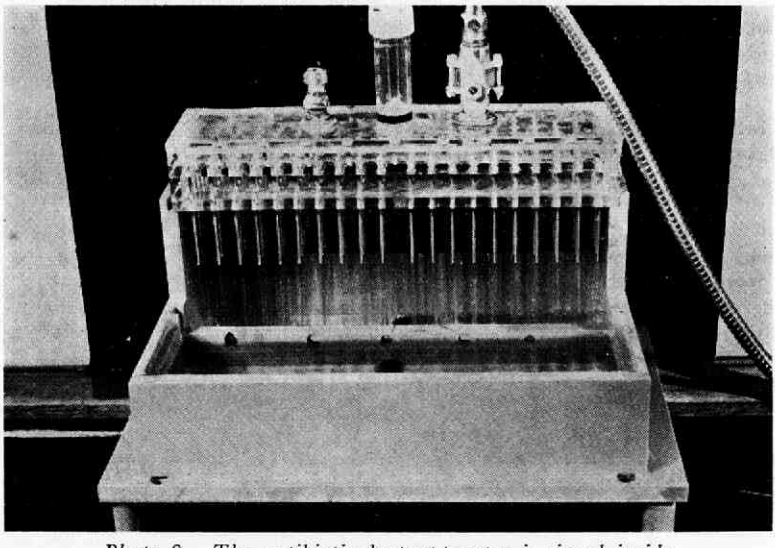


Photo 2. The antibiotic-drop-apparatus is rinsed inside.

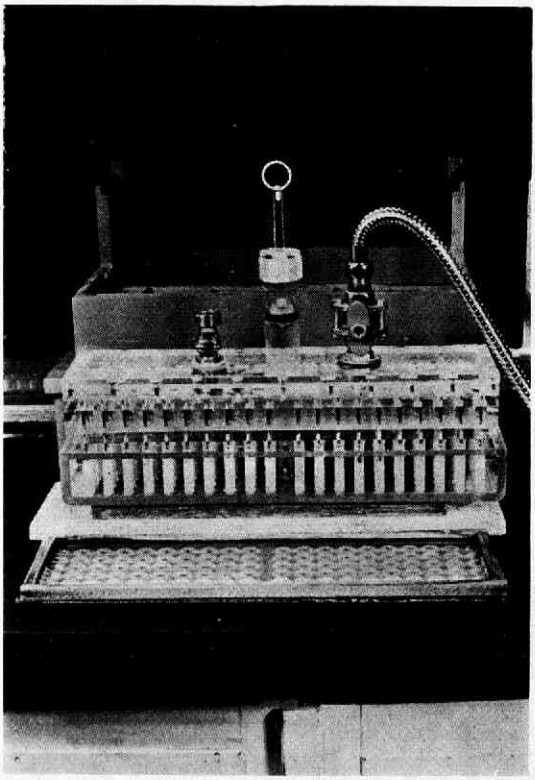


Photo 3. Three drops of milk are sucked by the antibiotic-drop apparatus out of the tubes placed in the tube-container.

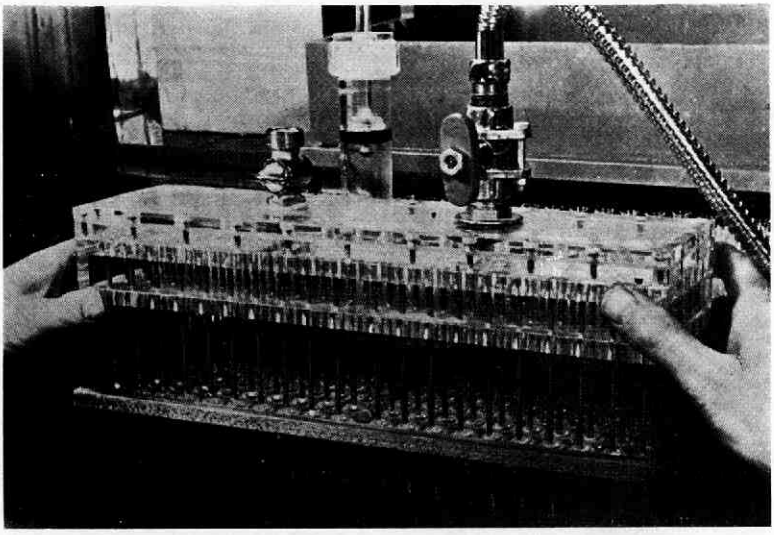


Photo 4. Three drops of milk are pipetted in 100 holes.

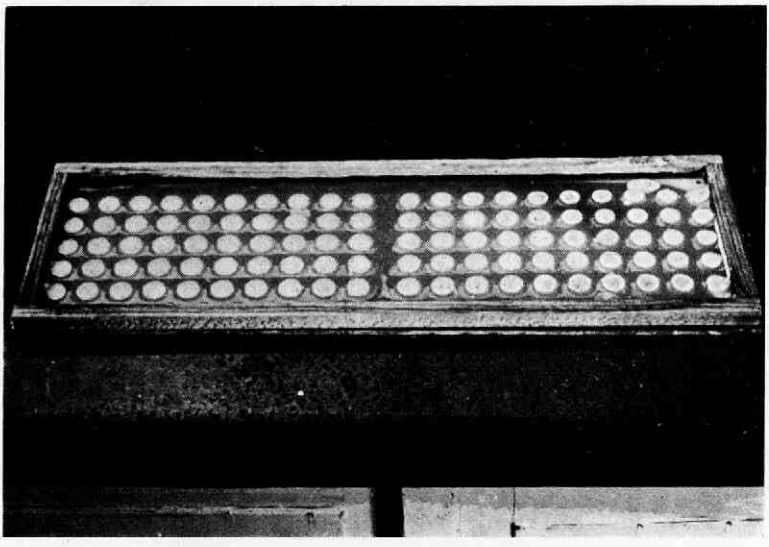


Photo 5. 100 holes are filled with milk.

Examination of milk samples on bacteria and inflammation-cells.

by F. H. J. JAARTSVELD*).

A. Bacteriological examination.

With the help of a platinum wire 1/50 ml. of milk is taken out of a sterile, uncentrifuged milksample and transferred to a blood and H.E.T.-medium, which now is incubated at 37° C for about 20 hours.
A rectangular collapsible plate was designed for examination by culture of milksamples taken under sterile conditions. The rectangular plate will be replaced in future by a plastic one, which will be used only one time. This plate is suited also for the detection of antibiotics in milk. The plate is divided into two unequal parts by a threshold running lengthwise.
Five to ten percent blood agar is poured into the left (greater) half and H.E.T.-medium into the right (smaller) half of the plate. The samples of milk to be examined are inoculated very simply and quickly on the two media in the order of the numbers marked on the threshold. The following day — after incubation — it is possible to determine whether any growth has occurred and the species of bacterium concerned.
The cultures of bacteria obtained from the milksamples can then also rapidly be examined for their sensitivity to penicillin and terramycin. Long strips of filterpaper containing a standardized quantity of these antibiotics are used for this purpose. For that purpose filterpaper "Whatmann 3" is used. The length is 300 mm and the breadth 2 mm. For ¾ of an hour they are sterilized at 120° C. Afterwards 0,225 ml. of a standardized penicillin and terramycin solution is spread over the whole strip. The first solution contains 1200 I.U. pen/10 ml. aqua-dest, the second solution contains 6000 I.U. terramycin/10 ml. aqua-dest.

B. Brabant Mastitis Reaction (B.M.R.).

After transferring the milksamples, the B.M.R. is carried out. To 0,6 ml. milk, 0,4 ml. sodium laurylsulfate 2% pH 12 is added.
After a good mixing the flow-through time is checked, after 5, 10, 20 and 60 seconds, and respectively indicated with B.M.R. —, •, ••, •••, ••••.

Flow-through time	B.M.R.	Total cells approximately
< 5 seconds	—	200.000
5 seconds	•	400.000
10 seconds	• •	800.000
20 seconds	• • •	2.000.000
60 seconds	• • • •	8.000.000

Determination of the bacteria.

Before the culture media are examined, we first look with the aid of an ultra violet lamp whether there is any reaction with aesculine on the H.E.T.-media.

*) F. H. J. Jaartsveld D.V.Sc.; veterinary surgeon to the Animal Health Service of North-Brabant; Rechterstraat 80, Boxtel; The Netherlands.

All bacterial colonies that decompose aesculine to aesculitine are black under U.V. light, and are marked on the culture disk.
With the aid of the following survey, the bacteria are determined.

Table 1.

	Blood medium		H.E.T. Medium		
	Growth	Hemolysis	Growth	Hemolysis	Aesculine decomposition
Strept. agalactiae	+	—(±)	+	+	—
Strept. dysgalactiae	+	—(±)	+	—(±)	—
Strept. uberis	+	—(±)	+	—(±)	+
Strept. lactis	+	—(±)	+	—(±)	+
Strept. faecalis	+	—(±)	+	—(±)	+
Strept. zoöepidemicus	+	++	++	++	—
C. pyogenes	±	+	—		
Staphylococci	++	+—	—(±)	—(±)	—
E. coli	++	+—	++	+—	—

The determination of *Streptococcus agalactiae* does not give any trouble with this method.
Streptococcus dysgalactiae is characterized by its flat bacterial colonies on the H.E.T. medium. To check this determination the culture is transferred to a horse-serum-agar medium. After the bacterial colonies have grown, a turbidity arises under the colonies.
The aesculine-decomposing streptococci, which were already marked on the culture-disks, cannot be determined by this scheme only.
Streptococcus faecalis mostly gives a greenish discoloration on the blood medium (alpha-hemolysis).

For further determination aesculine decomposing bacteria are transferred to media named in table 2.

Table 2.

	Str. lactis	*Str. faecalis*	*Str. uberis*	*Str. bovis*
Inuline	—	—	+	±
Raffinose	—	—	—	±
Sorbitol	—	+	+	±
Glycerol	—	+	—	—
Xylose	±	±	—	±
Litmus-milk	coagulation acid, reduction	reduction acid, coagulation	no coagulation acid, reduction	usually coagulation acid, reduction
Aesculine	+(±)	+	+	+

In extra-ordinary cases *Listeria monocytogenes* can be isolated. This bacterium also decomposes aesculine. However it is a Grampositive small rod, it decolorizes the litmusmilk (a so called white foot). Further examination is asked then.

Also the determination of *Streptococcus zoöepidemicus*, — that appears rarely — gives no problems.

Corynebacterium pyogenes has grown very badly after one day on the blood medium. On the H.E.T. medium it does not give any growth. This bacterium is mostly found in very abnormal milk. It is recommended to transfer directly, very abnormal milk on a Löffler medium.

C. pyogenes liquefies the Löffler medium in places where the bacterial colonies have grown; there a bit brown-coloured excavation arises. For further determination we make a Gram staining of the bacterial film. Then the bacterium is transferred to the determination sugars.

Staphylococci. These are growing luxuriantly on the blood medium, they are catalase positive, in contrast with the streptococci.

Staphylococci are usually found on the skin. They are called pathogenic in mastitis research when the B.M.R. of the milk is ••• or ••••.

Escherichia coli is also marked as a cause of mastitis, when the growth is accompanied by a positive B.M.R. In that case the milk is very abnormal.

Table 3.

	Escherichia coli	*Aerobacter aerogenes*[1])	*Pseudomonas aeruginosa*	*C. pyogenes*[2])	*Listeria*[3])
Motility	motile	non motile	motile	non motile	motile (22° C)
Glucose	acid + gas	acid + gas	no acid, or acid	acid ±, no gas	acid or neg.
Sucrose	neg. or acid + gas	acid + gas in 10 days	negative	acid ±, no gas	no acid, or acid, no gas
Lactose	acid + gas	acid + gas	negative	acid ±, no gas	no acid, or acid, no gas
Indol formation	positive	negative	mostly neg.	negative	negative
Litmus-milk	acid, coagulation	acid, no coagulation	coagulated, alkaline, reducted and liquefacted	coagulated at the bottom, acid formation after 48 hours; then peptonisation	reduction (white foot)
Serum broth	turbid	slimy ring	pellicle, greenish discolouration	sediment	sediment 37° C. turbid (22° C.)
Serum agar	luxuriant growth, grey bacterial colonies	slimy bacterial colonies, smell of trimethylamine	luxuriant growth, greenish coloration	very small bacterial colonies	small bact. colonies
Gelatine	no liquefaction	no liquefaction	liquefaction	liquefaction	no liquefaction
Löffler	no liquefaction	no liquefaction	slow liquefaction	liquefaction	no liquefaction

[1]) *Aerobacter aerogenes* resembles in proporties *Klebsiella pneumoniae* type B. (animal pathogenic form). This forms ammonia from pepton, so that the „slant" in the T.S.I. often becomes red after 2 x 24 hours, this is also the case with the yellow bacterial colonies on the Kauffmann medium.

[2]) *C. pyogenes* grows badly into the litmus-milk, it grows well after adding a little bit of liver-broth.

[3]) *Listeria* is catalase positive.

In other cases, the growth must be seen as defilement. For further determination this bacterium is placed on the media mentioned in table 3.

Culture media.

1. Meat-extract Broth.

aqua dest	1000 gr.
Liebig meat-extract	10 gr.
Bacto-pepton	5 gr.
Sodium chloride	5 gr.
Glucose	1 gr.

This solution is boiled for 10 min. on a open flame, cooled and filtered. The pH is adjusted to 7.3 and now sterilization for 10 minutes at 120° C. takes place. After cooling, filter through double paper till it's clear, and dispense the broth in flasks. These now are sterilized for 20 minutes at 100° C. The broth has to be clear. After cooling 10% sterile serum is added to the meat broth.

2. Serum agar.

250 ml. meat extract broth.
2% Bacto Agar = 5 gr.

Dissolve in the Koch-apparatus. Adjust the pH to 7,3. Sterilize in autoclave for 15 minutes at 110° C. Cool down at about 40° C. Add 10% horse-serum, sterile. Rotate carefully (no formation of bubbles), pour sterile in Petridiscs.

3. Blood Agar mdium.

To 250 ml. broth 2% Bacto-Agar is added.
To dissolve the agar well, it is placed during one hour in the Koch apparatus. Then it is sterilized for 15 minutes at 120° C. After cooling down at about 50° C., add under swinging about 25 ml. cow-blood, (defibrinated or citrateblood) and then the plates can be poured.
The citrateblood is prepared by adding ± 800 ml. cow-blood to 50 ml. of sterile 10% sodium citrate solution.

4. H. E. T. medium.

Aqua-dest 750 ml.
Tryptose agar 45 gr.
Aesculine 0,1 gr.
Fresh 1% thalliumacetate solution 33 ml.
Add aqua-dest till a volume of 1000 ml.

For a good solution the agar is placed for about 1 hour in the Koch, and then approximately 250 ml. are filled in bottles, during 20 min. sterilized at 120° C. After cooling down at about 45° C. with good mixing add 35 ml. cow-blood with crystal violet (C.D.I.)[1]) and 0,5 ml. B. toxine (C.D.I.) per 500 ml. agar.
Now the plates can be poured, after coagulation they are wrapped in brown paper, to protect this medium against light, which produces hemolysis.

5. Sugars.

a. Foundation solution (T. B. C. broth).

Tap water	1000 ml.
Tryptose	15 gr.
Yeastextract	0,2 gr.

[1]) Central Veterinary Institute, Prof. Poelslaan, Rotterdam.

L. cystine	0,2 gr.
Sodium-sulfite	0,1 gr.
Sodium-chloride	2,5 gr.
Secundair sodium phosphate	1,5 gr.
0,2 % phenolred solution in aqua-dest	50 ml.

For a good solution all the ingredients are boiled together for about 10 minutes and after cooling down the pH is adjusted to 7,3. Filter now and sterilize for 10 minutes at 130° C. After cooling down filter the turbid solution up to clear.

b. Sugar series.

10% sugarsolution in aqua-dest	10 ml.
Foundation solution	90 ml.

Pour in culture tubes with a little Durham tube. The sugars are now placed in the Koch, so that at the same time the air-bubbles in the Durham tubes, are driven away.

6. Litmus-milk medium.

1 litre skimmed milk is boiled and filtered.
Add to this milk 10% litmus-solution of Kubel and Tieman (BsS), filter. Pour culture tubes about 7 cc. per tube. Sterilize twice for ½ hour in the Koch, with an interim of 24 hours. (By sterilizing during a longer time the milk is discoloured). Instead of skimmed milk one can also use the solution: Skim-milkpowder 20 gram in 200 ml. aqua-dest).

7. Löffler medium.

3 parts cow-or horse-serum.
1 part meat-extract-broth.
1 to 2% glucose.

After mixing well and dissolving without heating the pH is adjusted to 7,6. The solution is poured into sterile culture tubes about 5-6 ml. These tubes are placed during 3 hours on 2 successive days in the serum-coagulation warmer at a temperature of 85° C.

8. Gelatine medium.

Aqua-dest 1000 ml.
Gelatine (trademark: two towers) 150 gr.

Warm up for ½ hour in the Koch at 100° C., then adjust the pH at 7,5—7,6. Filter through a „faltenfilter" till it's clear. Pour culture tubes, and sterilize on 2 successive days at 100° C. during ½ hour. Then cool it down, so the tubes coagulate in upright or sloping position.

REFERENCES

Jaartsveld, F. H. J.: Massaal bacteriologisch onderzoek van melkmonsters voor een georganiseerde mastitisbestrijding bij runderen. *Tijdschr. Diergeneesk.*, 87, *1088, (1962).*

SAMENVATTING.

A. **Bacteriologisch onderzoek.**

Op deze demonstratie wordt de rechthoekige schaal vertoond waarmee het mogelijk is het bacteriologisch onderzoek van een groot aantal melkmonsters in een korte tijd uit te voeren.
Deze schaal wordt in de lengterichting door een drempel in twee ongelijke delen verdeeld. In de grootste helft wordt 5-10% bloedagar en in de kleinste helft wordt

het H.E.T.-medium gegoten. Volgens de aangebrachte nummering wordt de plaat met de te onderzoeken melkmonsters op beide media geënt. De bacterie-culturen, afkomstig uit de melkmonsters, kunnen vervolgens op een snelle wijze onderzocht worden op hun gevoeligheid voor antibiotica zoals penicilline en terramycine. Dit gaat met behulp van lange strips van filtreerpapier, die een gestandariseerde hoeveelheid van het antibioticum bevatten.

B. **Brabantse Mastitis Reactie (B.M.R.).**

Aan 0.6 ml te onderzoeken melk wordt 0,4 ml Na-laurylsulfaat 2% (pH 12) toegevoegd. Dit wordt goed gemengd en overgebracht in z.g. doorstroomcapillairen. De tijd die dit mengsel nodig heeft om vanuit de trechter via de capillair uit te stromen, is een maat voor de slijmigheid van het mengsel, die op haar beurt een maat is voor het aantal cellen dat bij benadering in de melk aanwezig is.

Tijd voor doorstroming	B.M.R.	Benadering van het aantal cellen per ml melk
<5 sec.	—	200.000
5 sec.	•	400.000
10 sec.	• •	800.000
20 sec.	• • •	2.000.000
60 sec.	• • • •	8.000.000

Betreffende de determinatie van de bacteriën en de te gebruiken voedingsbodems worden uitgebreide voorschriften gegeven.

Yoghurt-, butter- and cheese inspection.

by H. J. BANNENBERG*)

Demonstration on „Campina-dairy" at Eindhoven and on the laboratory of the Provincial Animal Health Service at Boxtel.

In order to get an impression about the difference in quality of dairy-products made from s.c. "mastitis-milk" and normal milk, the following investigations are performed.
By means of the Brabantic Mastitis Reaction (B.M.R.) at the Campina-dairy, the supplied milk is divided in normal milk and mastitis-milk. Milksamples are taken out of all cans and examined by the B.M.R. The milk out of the cans with a negative B.M.R. is collected in one tank, the milk out of the cans with a positive B.M.R. (three or four points) is collected in an other tank. In this way mastitis-milk means milk of which 15-20% is drawn from abnormal quarters.
After working up the daily milk, about 4000 kg is pumped separately into two different buffertanks and is separated by two different and clean cream separators into two kinds (mastitis and normal for control) of cream (15 to 20% fat) and skimmed milk, leaving behind different quantities of separator slime.
Both kinds of cream are pasteurized in the usual way, soured and churned in a normal churn into butter and buttermilk.

Recombined pasteurized milk was made from separated cream and skimmed milk for the yoghurt making in the usual manner.

Two kinds of milk collected in the same manner, were transported to the cheese-factory "Dongen". Two batches of cheese were made without passing the milk through the cleaning-separator.

As the first experiment suggested that there was a difference in copper content between mastitis-butter and normal or control-butter precautions were taken to prevent any copper contamination in the "milk-way" and routine samples of both kinds of milk and products were taken for examination of copper content by this institute and the laboratory of the Association of co-operative Dairying in the south of The Netherlands (C.Z.N.Z.) at Roermond.
Organoleptic and other tests were carried out by the laboratories of the C.Z.N.Z. and the N.I.Z.O. (Institute for Research in Dairies in The Netherlands) and the department of the Z.K.B. (Dairy Quality Control Bureau) at this town (Boxtel).

Discussion of results.

a. B.M.R. and cell-counts of milk; quantity of separator-slime.

Table 1 shows that the quantities of separator-slime vary with the kind and quantities of milk, but the mastitis-milk contains more than double the quantity of slime.

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Table 1.

Results of B.M.R., cell-counts and separator experiments at the Campina-dairy. Eindhoven.

Period:	Winter:		Summer:	
Date of selection:	15-11-'62	18-4-'63	27-8-'63	28-8-'63
Object of experiment:	buttermaking	cheesemaking	buttermaking	cheesemaking
B.M.R. M-milk	• • •	• • •	• • •	• • •
N-milk	—	—	—	—
Cell-count M-milk	not determined	1.500.000	1.300.000	1.275.000
N-milk	not determined	not delivered	90.000	120.000
Estimated quantity of both milks	2.800 kg	5.500 kg	4.000 kg	4.000 kg
Quantity of seperator slime M-milk	1.120 gr	not separated	1.335 gr	not separated
Quantity of seperator slime N-milk	160 gr	not separated	533 gr	not separated

b. Yoghurt and buttermilk.

Both fresh and after 5 days keeping in the refrigerator no differences were found in the organoleptic tests of the mastitis and controlproducts, when these were made of recombined milk or separated and soured cream.

There appeared to be some difference in taste (unfresh, unclean, salty), when these products were made directly of pasteurized (not cream separated) of naturally skimmed cow milk. You can try this in an organoleptic test afterwards. Up till now, no differences were found in copper content of the routine samples.

c. Butter.

The results of the organoleptic and other tests of the keeping quality of mastitis and controlbutter of the first experiment dated 15/11 1962 are summarized in table 2 and table 3.

Table 2.

Classification quality of mastitis and controlbutter in cold storage according to Z.K.B. (three samples)

Age of the samples:	14 days	1 month	2 month	3 month	4 month	5 month
M-butter	I —	I	I —	II fatty	III bacony	III train oily
	I	I	I	II fatty	III bacony	III train oily
Mastitis	I —	I	I —	II fatty	IV bacony	III train oily
C-butter	I —	I	I —	I —	I —	I —
	I	I	I	I —	I —	I —
(control)	I —	I	I	I —	I — à II slightly fatty	I —

The first results of the second experiment dated 27/8 1963 are summarized in table 4.

From tables 2 and 3 can be concluded that the mastitisbutter of the first experiment (winterperiod) in cold storage spoiled quicker than the controlbutter. Whether this is due to the difference in coppercontent is still uncertain.

Table 3.

Keeping quality of mastitis and control butter according to N.I.Z.O.

Age of the samples:	14 days	1 month	2 month	3 month	4 month	5 month
Organol test M-Butter	sl. fatty	sl. fatty	train-oily	very train-oily	very train-oily	very train-oily
C-Butter	sl sour sl. fatty	sl. fatty	fatty	fatty	fatty	fatty sl. train-oily
T.B.A.-test						
M-butter:	0,054	0,074	0,236	0,313	0,536	0,550
C-butter:	0,083	0,058	0,102	0,082	0,148	0,190
Peroxide-number						
M-butter:	0,00	0,00	0,40	0,66	0,56	0,84
C-butter:	0,00	0,02	0,32	0,19	0,24	0,32
Sour-number						
M-butter:	0,59	0,59	0,56	0,60	0,64	0,58
C-butter:	0,64	0,65	0,67	0,68	0,74	0,68
Copper-content						
M-butter:	82 microgram/kg of butter					
C-butter:	46 microgram/kg of butter.					

Table 4.

Coppercontents of mastitis and controlbutter according to C.Z.N.Z. and N.I.Z.O.

M-butter:	I	35 microgr. Cu/kg	Average C.Z.N.Z.:	N.I.Z.O.:
	II	40 microgr. Cu/kg	45 microgr./kg	44 microgr./kg
	III	60 microgr. Cu/kg		
C-butter:	I	20 microgr. Cu/kg		
	II	35 microgr. Cu/kg	27 microgr./kg	37 microgr./kg
	III	25 microgr. Cu/kg		

The first results of the second experiment (summerperiod; 27-8-'63) give copper contents, which are absolutely lower, though in the same direction (table 4). Other factors can also influence the keeping quality of butter in cold storage.

For example Van Duin found *(Off. Org. F.N.Z., 53, 331, (1963))* that traces of blood in a concentration of 1:10.000 already are harmful. According to the Z.K.B. butter with a copper content above 70 microgr./kgr. in the summerperiod and above 100 microgr./kgr. in the winterperiod is not suited for cold storage.

d. Cheese.

For the first cheese-experiment dated 18/4 1963, only mastitismilk was delivered and therefore no direct comparison could be made.

An organoleptic test of both kinds of cheese of the second experiment dated 28/8 1963 after only 3 weeks showed that the mastitischeese had a slightly sour taste, a doughy consistence, a sticky rind and irregular holes, while the controlcheese was normal, as you can test in an organoleptic test afterwards. We had the impression, that the souring was retarded. Other factors are under examination.

Conclusions.

1. By means of the B.M.R. it is possible to separate at a dairy-factory s.c. mastitis-milk from normal milk.
2. Mastitis-milk collected by this way has a high cell-count (about 1.500.000 cells per ml.); the quantity of separator slime is much more than the quantity of separator slime from normal milk.
3. Yoghurt and buttermilk made from s.c. mastitis-milk and normal-milk, both fresh and after 5 days keeping in the refrigerator, show no differences in the organoleptic tests.
   There appeared to be some difference in taste (unfresh, unclean, salty), when these products were made directly of pasteurized (not cream-separated) or naturally skimmed cow-milk.
4. There seems to be a difference in the quality of butter made from s.c. mastitis- and normal-milk, especially when the butter has been stored for some months. The butter made from mastitis-milk deteriorates quicker.
5. There appeared to be a difference in taste, consistency and structure of cheese made from the two kinds of milk. Cheese made of mastitis-milk was of a lower quality.
6. It is very important to repeat investigations with more controls than we have done here, in order to get a clear insight of these problems.

SAMENVATTING.

Op de zuivelfabriek „Campina" te Eindhoven werden enkele malen „mastitismelk" (celrijke) en controle melk (celarme) verzameld.
Daartoe werd van elke bus een monster genomen en ter plaatse volgens de B.M.R. onderzocht. De melk, afkomstig uit bussen met een positieve reactie, werd in een aparte tank gestort, terwijl in een andere tank de „negatieve" bussen werden geleegd. Beide groepen bevatten dus melk van dezelfde bedrijven.
Het gelukte zo 3000 à 4000 kg „mastitismelk" te verzamelen, waarbij kan worden vermeld dat 15 à 20% van deze melk afkomstig is uit ontstoken kwartieren. Het aantal cellen in de controlemelk bedraagt dan ± 200.000 per ml terwijl de „mastitismelk" er ruim een miljoen per ml bevat.
Bij organoleptische keuring bleek yogurt en karnemelk, gemaakt van „mastitismelk" van mindere kwaliteit (onfris-zoutig) dan dezelfde produkten gemaakt van controlemelk. Dit verschil viel echter weg, indien de melk eerst gecentrifugeerd werd en dan door menging van room en ondermelk opnieuw werd samengesteld. Bij centrifugeren bleek, dat de mastitismelk belangrijk meer centrifugeslib gaf dan de controlemelk. Boterpartijen, gemaakt van beide soorten melk, vertoonden in verse toestand geen organoleptische verschillen. Bij bewaring in het koelhuis traden er na 2 à 3 maanden kwaliteitsverschillen op. De boter gemaakt van mastitismelk werd dan vettig, spekkig, tranig. Het viel op dat de „mastitisboter" meer koper bevatte dan de controleboter. Dit was bij de eerste proef (winter) duidelijker dan bij de tweede proef (zomer). De keuringen vonden plaats door de CZNZ (Roermond), het NIZO (Ede) en ZKB (Boxtel).
Op de zuivelfabriek te Dongen werd kaas gemaakt van resp. mastitismelk en controlemelk. Deze melk was niet gecentrifugeerd. Ook deze beide partijen melk waren op „Campina" te Eindhoven verzameld. Na enige weken bleek de mastitiskaas wat deegachtig en wat nestig, de korst was week. De kaas, gemaakt van controlemelk, was normaal van kwaliteit. Gedacht wordt aan een vertraagde zuring als oorzaak van deze verschillen.
Het is van groot belang dat deze proeven herhaald worden, opdat door een nadere bestudering een beter inzicht verkregen wordt in deze problemen.

Closing of the Conference

Danksagung.

von K. TILGNER*)

Meine Damen und Herren.

Darf ich mal einige Worte an Herrn Kollege Brus sprechen. Ich möchte, auch in Ihren Namen, lieben Herren Kollegen, unseren Dank aussprechen für die Einladung zu diesem Kongress über Mastitis-Frage.
Ich glaube, dass diese Tage sehr erfolgreich waren und dass sie uns allen viel Nutzen gebracht haben.
Zugleich möchte ich Sie bitten, Dr. Brus, auch dem Vorstand Ihres Provinzialen Gesundheitsdienstes unseren Dank auszurichten.
Dass wäre dann, was ich zu sagen hatte.

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Closing address.

by D. H. J. BRUS*)

Ladies and Gentlemen,

At the close of this congress, I wish to speak a few more words.
I believe that I may say that we have had a successful congress. We have listened to many speakers and we have heard various views, particularly during the last discussions, and I am glad that they differ.
I hope that these questions will induce everyone to start investigating. An investigation will only be a good investigation if it supplies the answer to a question and at the same time raises two fresh questions which have to be answered.
I hope that the various investigators will test one another's methods and that they will write personally on the results obtained or, perhaps even better, personally discuss these results.
May the personal contacts we have now established bear fruit in the practical work of mastitis control.
I am glad that you have attended these congress days and I cordially thank you for doing so.
All of you who have come from Finland, Sweden, Norway, Denmark, Germany, Great Britain, Belgium, the United States of America and, last but not least, The Netherlands.
A special word of thanks is due to the speakers Dr. Schalm, Prof. Van der Schaaf, Mr. Kingwill, Dr. Jaartsveld, Mr. Cazemier, Dr. Scheiner and Dr. Richter.
Thanks also to Prof. Vandeplassche and Dr. Tilgner who conducted the discussions.
I also wish to thank the management of the Co-operative Dairy Works "Campina" as well as our own committee, who have enabled Dr. Jaartsveld and myself to organize this congress.
I hope that this congress will lead to many contacts in the future and wish you all a pleasant journey home.

Good-bye,
Auf Wiedersehen,
Au revoir.

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