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TRACER DYE GREEN S (FOOD GREEN NO. 4) IN PENICILLIN PREPARATIONS FOR INTRAMAMMARY APPLICATION

III. CONTAMINATION OF BULK MILK WITH PENICILLIN FOLLOWING USE OF INTRAMAMMARY PREPARATIONS WITH AND WITHOUT TRACER DYE¹⁾

By

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In a preliminary work *Dalgaard-Mikkelsen & Rasmussen* (1957) studied the usefulness of Food Green No. 4 as a tracer dye for detecting antibiotics in milk after intramammary application, the dye being incorporated in the vehicles of the preparation concerned.

That work was followed up by *Rasmussen & Simesen* (1960), who determined the optimal amount of dye per dose, and also demonstrated the utility of the dye as tracer for penicillin preparations with varying excretion times. *Høgh & Rasmussen* (1961) have used Food Green No. 4 with satisfactory results for preparations with short excretion times.

The aim of the present study was to examine under practical conditions the extent of contamination of bulk milk by penicillin after use of intramammary preparations with and without tracer dye.

MATERIAL

The study, which was been carried out over a period of six months, comprised two milk depots with 32 and 71 suppliers respectively.

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As *routine control*, samples were taken from the bulk milk from all the suppliers on days selected at random. In addition, samples were taken during about three months from the tank lorries which brought supplementary milk from nine creameries.

The control of milk from *herds under treatment* included 63 herds in which acute and chronic cases of mastitis were being subjected to intramammary treatment with the following preparations: —

- A. 14 herds: Commercial products with varying excretion times. The suppliers were instructed to retain the milk from the treated quarters for four days.
- B. 19 herds: Experimental products¹⁾ with a maximum excretion time of 72 hours: —
 - (1) Procaine benzyl penicillin in hydrophilic vegetable oil (viscoleo), 1 ml. = 50,000 i. u.
 - (2) Procaine benzyl penicillin + dihydrostreptomycin sulphate in hydrophilic vegetable oil (viscoleo), 1 ml. = 50,000 i. u. penicillin + 100 mg. DHS.
- C. 30 herds: Experimental products (1) and (2) with 50 mg. Food Green No. 4 added per 100,000 i. u. penicillin.

As regards B. and C., the suppliers were instructed to withhold the milk from the treated quarters for three days.

Sampling was commenced on the day after treatment and was continued up to five days after the last treatment in the cases where one of the experimental preparations was used, and up to six days after the last treatment when one of the commercial preparations was used.

In the herds where there was *acute mastitis*, the veterinary surgeon (*W. N. Lynggaard*, Veksø) planned the treatment according to the type of case in question. Generally the treatment consisted of two applications with an interval of about 48 hours; only in a few cases was one application sufficient. The dosage employed varied from 100,000 to 300,000 i. u. per quarter.

Re-treatment of herds with *chronic streptococcal mastitis* (B, C, G or L streptococci) was carried out in all cases about 48 hours after the first treatment. With one exception only, all four quarters were treated regardless of the intensity of the in-

¹⁾ The preparations used were kindly supplied by NOVO Therapeutic Laboratory, Copenhagen.

fection in the cows concerned. The dosage employed varied from 100,000 to 200,000 i. u. per quarter.

In those herds where cases of *clinically recognizable milk changes* were demonstrated on routine sanitary inspection, the cows concerned were treated with 100,000 to 150,000 i. u. of the experimental preparation per quarter. Only quarters with abnormal secretion were treated.

METHODS

The milk specimens were all examined for bacteriostatic substances by being allowed to drip undiluted on to *Sarcina lutea* agar cup plates. All the positive specimens were controlled with penicillinase, and only if no inhibition zone could be seen on addition of penicillinase was the bacteriostatic substance regarded as penicillin.

Penicillin determination was carried out by the extraction technique with butyl acetate described by *Hallas-Møller, Juncher, Møller & Wille* (1952), by which method 0.002 i. u. of penicillin per ml. can be demonstrated. The same amounts of milk, butyl acetate and buffer were used for extraction as indicated by *Høgh & Rasmussen* (1961).

Specimens originating from suppliers where treatment was carried out with experimental preparations with Food Green No. 4 added were also examined for content of tracer dye by means of the following technique: —

50 ml. milk is drawn by means of suitable vacuum for about two minutes through an approximately 10 mm. high (diameter 8 mm.) ion exchange column (Dowex No. 1 × 4, 100/200). If the specimen contains Food Green No. 4 the upper part of the column becomes green to a depth dependent on the concentration of Food Green No. 4 in the milk. By this method it is possible to demonstrate a content of Food Green No. 4 in the milk of 0.03 µg. per ml., which corresponds to a penicillin content of 0.002 i. u. per ml.

RESULTS

Random sampling

Examination for penicillin of bulk milk from all herds

Specimens were taken two and four times, respectively, from all the cans in which milk was transported from the suppliers to the two depots. The results are assembled in Table 1, from which it can be seen that control analysis on days selected at

Table 1. Number of suppliers of milk containing penicillin and number of contaminated cans delivered, determined by routine examination of all transport cans.

No. of suppliers	No. of cans	No. of suppliers of milk containing penicillin	No. of cans containing penicillin	Penicillin concentration i. u. per ml.
32	100	1	1	0.043
71	290	3	7	0.003—0.15
32	118	2	3	0.002—0.007
71	292	3	8	0.007—0.016
71	318	1	6	0.008—0.02
71	269	0	0	0.00

random revealed penicillin in the milk from 0 to 3 per cent. of the cans. The concentrations were generally small, though such large amounts as 0.15 i. u. per ml. could be found.

In conjunction with these routine examinations, samples were taken again during the following days from all cans from suppliers who had delivered milk containing penicillin. Hereby it was sometimes found that the supply of milk containing penicillin continued during the next two or three days. In one case of a supplier whose milk very often contained penicillin and who, by virtue of his occupation, had free access to antibiotics, samples were taken twelve times in the course of three weeks, and penicillin was demonstrated in 32 out of a total of 84 cans.

Penicillin in tank milk

Out of 574 specimens taken from tank lorry deliveries of supplementary milk received by the two depots from creameries, 7 were found to be contaminated with penicillin in concentrations varying from 0.002 to 0.013 i. u. per ml. The amount of milk in the tanks was 2,000 to 3,000 litres and the largest total content of penicillin in one tank was 26,000 i. u.

Penicillin in specimens of bulk milk from treated herds

The herds under treatment for mastitis could be divided into three groups according to the preparations used for treatment. As will be seen from Table 2, 7 out of 14 suppliers in whose herds commercial products with varying excretion time were used (group A) delivered milk containing penicillin during the specifi-

Table 2. Number of suppliers of milk containing penicillin and number of contaminated cans, determined by examination of all cans of milk from herds treated with commercial products (A), experimental preparations (B), or experimental preparations with Food Green No. 4 added (C).

Preparations	Total	No. of suppliers of milk containing penicillin		Number of cans			
		In retention period	After retention period	In retention period		After retention period	
				Total	Containing penicillin	Total	Containing penicillin
A. Commercial products	14	7	3	333	38	140	6
B. Experimental preparation 1 or 2	19	9	0	210	24	83	0
C. Experimental preparation 1 or 2 with tracer dye added	30	4	0	643	5	354	0

ied retention period (4 days) and milk from three herds also contained penicillin after expiry of the retention period. Within this group, 38 out of 333 transport cans contained penicillin during the retention period and 6 out of 140 after expiry of the period.

Where experimental preparations with short excretion times were used (group B), the tendency to deliver milk containing penicillin during the retention period (3 days) was much the same, since 9 out of 19 suppliers delivered milk in which penicillin could be found. In this group 24 out of 210 transport cans contained penicillin during the retention period. On expiry of the period, however, no penicillin could be found in the milk.

In group C the effect of the addition of tracer dye could be seen. Out of 30 suppliers, only 4 delivered milk containing penicillin during the retention period (3 days) and only 5 out of a total of 643 transport cans were found to be contaminated. On expiry of the retention period no penicillin was found in the milk. It should be noted that in two of the five contaminated cans, where the concentrations were 0.006 and 0.002 i. u. per ml. respectively, no tracer dye could be seen — a fact which will be discussed later.

Table 3 shows the suppliers who delivered milk containing penicillin during the retention period, stating the amount of their daily average total deliveries, how many litres of milk containing

Table 3. Intensity and distribution of penicillin contamination (A), experimental preparations (B), or experimental

Average daily supply, litres	1st day			2nd day		
	Contaminated milk, total in litres	Penicillin, i. u./ml.	Penicillin, total i. u.	Contaminated milk, total in litres	Penicillin, i. u./ml.	Penicillin, total i. u.
375	100	0.003	300	100	0.005	500
200	0	0	0	0	0	0
200	0	0	0	0	0	0
A 200	50	0.006	300	100	0.002	200
350	0	0	0	50	0.013	650
225	0	0	0	190	0.002	380
100	0	0	0	0	0	0
275	90	0.070	6300	40	0.004	160
250	40	0.092	3680	40	0.003	120
220	40	0.004	160	200	0.004	800
210	50	0.056	2800	0	0	0
B 190	130	0.003	390	0	0	0
100	25	0.002	50	0	0	0
180	0	0	0	50	0.013	650
225	50	0.004	200	0	0	0
110	0	0	0	25	0.057	1425
230	0	0	0	90	0.009	810
C 160	25	0.006	150	0	0	0
280	0	0	0	0	0	0
200	0	0	0	50	0.002	100

1) Dihydrostreptomycin

2) Treatment repeated on 2nd day

— Delivery stop

penicillin were delivered by them, and the concentration of penicillin in the milk. It will be seen that, when ordinary commercial products were used (A), only two herds delivered milk contaminated with penicillin the first day after treatment, and the concentrations were small. On the second, third and fourth days after treatment, the frequency of contaminated deliveries and the extent of the contamination increased, and there was still considerable contamination on the fifth day.

After use of the experimental preparations which were easily blended with the milk and quickly excreted, the findings were quite different (B). On the first day after treatment, strongly contaminated milk was delivered from almost all the herds. By

during the first five days after treatment with commercial products preparations containing Food Green No. 4 (C).

3rd day			4th day			5th day		
Contaminated milk, total in litres	Penicillin, i. u./ml.	Penicillin, total i. u.	Contaminated milk, total in litres	Penicillin i. u./ml.	Penicillin, total i. u.	Contaminated milk, total in litres	Penicillin, i. u./ml.	Penicillin, total i. u.
100	0.005	500	50	0.002	100	0	0	0
100	0.012	1200	50	0.005	250	0	0	0
50	0.015	750	150	0.005	750	100	0.008	800
0	0	0	0	0	0	0	0	0
90	0.008	720	175	0.007	1225	150	0.007	1050
0	0	0	0	0	0	0	0	0
100	0.023 ¹⁾	2300	100	0.030 ¹⁾	3000	40	0.03 ¹⁾	1200
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
75	0.11 ²⁾	8250	150	0.16 ²⁾	24000	—	—	—
0	0	0	0	0	0	0	0	0
50	0.004	200	0	0	0	—	—	—
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
40	0.004	160	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0

the next day the frequency had decreased, and on the third day contamination was found in only one case (not taking into consideration one herd where treatment was repeated).

When Food Green No. 4 was added (C), there was no regularity in the findings of contaminated milk. The four suppliers delivered milk containing penicillin on one occasion each and at different times during the first three days after treatment. In all cases the concentrations were small.

No relationship could be demonstrated between the size of the herd and the tendency to deliver milk containing penicillin, nor did there seem to be any connection between the intensity of the treatment (dose and number of treated quarters in relation to the total number of quarters) and the degree of the penicillin contamination.

Influence of Food Green No. 4 on the therapeutic effect of intramammary penicillin preparations

Thirty-three cows with 55 quarters infected with haemolytic streptococci were examined bacteriologically both before and after treatment with the experimental preparation containing procaine benzyl penicillin and Food Green No. 4. With the exception of one case, all four quarters were treated and the treatment was repeated about 48 hours after the first application. Follow-up examination about a month after treatment showed that 26 cows and 46 quarters were clear, which corresponds to the results obtained by *Rømer* (1961) with the same preparation without Food Green No. 4.

DISCUSSION

When comparing the contamination rate found in the present study, where random sampling revealed the presence of penicillin in 25 out of 1,387 transport cans (1.7 per cent.) and in 7 out of 574 tank lorries (1.2 per cent.), with previously published Danish works, it must be borne in mind that the method used for demonstration of penicillin has a sensitivity of 0.002 i. u. per ml. *Malling Olsen* (1960) reports that the frequency of contamination with penicillin in 9,175 bulk milk specimens examined in the municipal laboratories was 0.3 per cent. with a limit of 0.02 i. u. per ml. Using a limit of 0.5 i. u. per ml. *Pilegård Andersen & Leth Jørgensen* (1959) found penicillin in the milk from about 0.06 per cent. of a total of 15,557 herds.

Comparison of the Danish findings with conditions in other countries shows that Norway — where, as in this country, there is strict control with the supply of intramammary preparations — with a percentage of milk contaminated with penicillin of 0.1 to 0.6 (0.003 i. u. per ml.) (*Ellingsen* 1961), — is, like Denmark, more favourably situated than countries where there is free access to intramammary preparations. *Naylor* (1960) states that in Australia the contamination is 3.2 per cent. (0.05 i. u. per ml.) and 9.7 per cent. (0.005 i. u. per ml.) respectively. *Berridge* (1956) reports the figure for Great Britain as being up to 18.5 per cent. (0.01 i. u. per ml.). In U.S.A. 3.7 per cent. (0.003 per ml.) of the tank milk examined contained antibiotics (*Jester, Wright & Welch* 1959). *Kosikowski* (1960) found that the contamination frequency for milk delivered from part of New York State was

reduced from about 5 per cent. to 0.5 per cent. as the result of a propaganda campaign.

With a measuring method with a sensitivity of 0.002 i. u. per ml., it is possible that some of the positive milk specimens may have originated from deliveries, permissible at the time of the examination, of milk from untreated quarters. As shown by *Livoni, Madelung, Pedersen & Westh* (1950), *Blobel* (1960) and *Snyder, Drury & Weaver* (1961), the first milking after intramammary application of antibiotics may reveal excretion from untreated quarters. Such finding of penicillin in milk from untreated quarters may possibly explain the fact that, as mentioned above, in two cases no tracer dye could be demonstrated in contaminated milk from herds where treatment had been given with, respectively, twice 100,000 and three times 250,000 i. u. penicillin in the experimental preparation with Food Green No. 4 added. This assumption seems to be reasonable when it is considered that *Dalgaard-Mikkelsen & Rasmussen* (1962) have proved that the ion exchange method for demonstration of tracer dye is absolutely certain with concentrations of 0.03 μ g. per ml. or more, which corresponds to a penicillin content of 0.002 i. u. per ml.

It is obvious from study of the material covering control of herds under treatment that the rules concerning retention of milk contaminated with antibiotics had been disregarded by some suppliers, even taking into account the reservation regarding the supply of milk from untreated quarters of treated cows. The difference in the time of commencement of unlawful delivery found between herds in which treatment was carried out with commercial products and those treated with non-stained experimental preparations is probably explained by the fact that at first milking a number of older commercial products with hydrophobic vehicles leave visible drops on the surface of the milk. This means that contamination is recognized and delivery therefore not commenced till late in the retention period. The experimental preparations which are hydrophilic are better distributed in the milk, which means that the tendency to commence delivery of milk from treated quarters on the first day after treatment is much greater.

The effect of the visual deviation of the milk from the normal colour is very clearly indicated by the marked decrease in the frequency of milk containing penicillin which was observed after

treatment with preparations to which Food Green No. 4 had been added. The strong green colour of the milk on the first milkings after application reminds the milker of the rules, thus preventing a number of omissions and mistakes which may arise when, for instance, relieving personnel take over the work. This is an interpretation which is supported by the statements of owners and milking staff in herds treated with experimental preparations containing tracer dye. In addition to ensuring rapid control of the most important source of contamination of milk with penicillin, viz. intramammary treatment, incorporation of tracer dye in the preparations will reduce the risk of unintentional delivery of milk containing penicillin.

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REFERENCES

- Berridge, N. J.*: Penicillin in Milk. II. The Incidence of Penicillin. *J. Dairy Res.* 1956, 23, 342.
- Blobel, H.*: Concentrations of Penicillin in Milk Secretions and Blood Serums of Cows Following Intramammary Infusion of One or More Quarters. *J. Amer. vet. med. Ass.* 1960, 137, 110.
- Dalgaard-Mikkelsen, Sv. & F. Rasmussen*: Røbefarve i antibiotikaholdige intramammaria. *Nord. Vet.-Med.* 1957, 9, 852.
- Dalgaard-Mikkelsen Sv. & F. Rasmussen*: Tracer Dyes for Rapid Detection of Antibiotics in Milk. XVI International Dairy Congress 1962. (in press).
- Ellingsen, J. K.*: Antibiotica i leverandørmelk. *Medlemsbl. norske Veterinærfor.* 1961, 15, 57.
- Hallas-Møller, K., H. O. Juncher, C. Møller & B. Wille*: Nogle kemiske og biologiske undersøgelser over et organspecifikt penicillin-derivat. Add. I: Analysemetode til bestemmelse af lungevævs og plasmas indhold af frit aktivt penicillin efter indgift af DAP (Leocillin) og prokainpenicillin. *Ugeskr. Læg.* 1952, 114, 447.
- Høgh, P. & F. Rasmussen*: Tracer Dye Green S (Food Green No. 4) in Penicillin Preparations for Intramammary Applications II. *Acta vet. scand.* 1961, 2, 185.
- Jester, W. R., W. W. Wright & H. Welch*: Antibiotics in Fluid Milk. *Antibiotics & Chemotherapy* 1959, 9, 393.
- Kosikowski, F. V.*: The Control of Antibiotics in Milk Through a Sound Test Program. *J. Milk Food Techn.* 1960, 23, 285.

- Livoni, P., P. Madelung, J. Pedersen & J. Westh:* Fortsatte syrningsforsøg med mælk fra penicillinbehandlede køer. Nord. Vet.-Med. 1950, 2, 591.
- Malling-Olsen, E.:* Mejerimæssige og folkesundhedsmæssige problemer i forbindelse med antibiotica i mælk. Nord. Mejeritidsskr. 1961, 27, 195.
- Naylor, J.:* The Incidence of Penicillin in Australian Milk Supplies. Austr. J. Dairy Techn. 1960, 15, 153.
- Pilegård Andersen, C. & K. Leth Jørgensen:* Undersøgelse over forekomst af penicillin og et af *Str. saccharolactis* produceret antibioticum i den på mejerierne modtagne mælk. Nord. Vet.-Med. 1959, 11, 316.
- Rasmussen, F. & B. Simesen:* Tracer Dye Green S in Penicillin Preparations for Intramammary Applications. Nord. Vet.-Med. 1960, 12, 120.
- Rømer, O.:* Personal communication, 1961.
- Snyder, W. W., A. R. Drury & E. Weaver:* Residues in Milk, Blood and Urine Resulting from Various Types of Antibiotic Administration. Mich. Agr. Expt. Sta. Quart. Bull. 1961, 43, 539.

SUMMARY

Herds with acute and chronic mastitis were treated with both ordinary commercial intramammaria and experimental preparations with and without tracer dye. The experimental preparations consisted of penicillin and a penicillin and dihydrostreptomycin mixture in hydrophilic vegetable oil. As tracer dye 50 mg Food Green No. 4 per 100,000 i.u. penicillin was used. The dose of penicillin used for treatment varied from 100,000 to 300,000 i.u. per quarter. Fourteen herds were treated with commercial products, 19 with experimental preparations, and 30 with experimental preparations containing tracer dye.

Specimens were taken occasionally from the milk cans from all suppliers and from the tank lorries which brought supplementary milk from other creameries. On the first day after treatment, can samples were taken from the herds concerned and examined for content of penicillin, and in those cases where experimental preparations with tracer dye were used, for content of colouring matter also. The tracer dye could be demonstrated in the course of about two minutes by means of an ion exchange technique which is sensitive to 0.03 μg dye per ml, corresponding to 0.002 i.u. penicillin per ml.

On random sampling, penicillin (≥ 0.002 i.u. per ml.) was found in up to 3 per cent. of all the cans delivered (Table 1) and in 7 out of 574 tank samples.

More than 10 per cent. of the transport cans were found to be contaminated with penicillin in the retention period when commercial products and experimental preparations without tracer dye had been used for mastitis treatment, while less than 1 per cent. were

found to be contaminated when experimental preparations containing Food Green No. 4 had been employed (Table 2).

The intensity and distribution of the penicillin contamination during the first five days after treatment varied (Table 3). Where commercial products were used, the contamination was greatest towards the end of the retention period, while in the case of the experimental products it was greatest at the beginning of the retention period. There was no regularity in the deliveries of milk containing penicillin where experimental preparations with tracer dye had been used, and the concentrations found were considerably less than those found after use of commercial products and experimental preparations without tracer dye.

Tracer dye did not seem to have any influence on the therapeutic effect of the preparations.

Incorporation of tracer dye in preparations for intramammary infusion ensures rapid testing of the milk delivered and, in addition, reduces the risk of unintentional admixture of milk containing penicillin.

ZUSAMMENFASSUNG

Die Anzeigefarbe Green S (Food Green No. 4) in penicillinhaltigen Intramammarien.

III. Die Penicillinkontaminierung von Liefermilch nach Anwendung von Intramammarien mit und ohne Anzeigefarbe.

Bestände mit akuten und chronischen Mastitiden wurden teils mit allgemein im Handel vorkommenden Mastitispräparaten, teils mit Versuchspräparaten mit oder ohne Anzeigefarbe behandelt. Als Versuchspräparate dienten Penicillin und Penicillin-Dihydrostreptomycin, mit hydrophilem vegetabilischem Öl vom Typ Viscoleo gemischt. Als Anzeigefarbe diente Food Green No. 4 in einer Menge von 50 mg pro 100 000 I.E. Penicillin. Die Penicillindosis variierte pro Behandlung von 100 000—300 000 I.E. pro Euterviertel. Mit Handelspräparaten wurden 14 Bestände, mit Versuchspräparaten 19 Bestände und mit Versuchspräparaten mit Zusatz von Anzeigefarbe 30 Bestände behandelt.

Gelegentlich wurden Kannenmilchproben aus sämtlichen Lieferungen und Proben aus den Tankwagen mit Ersatzmilch entnommen. Am ersten Tage nach der Behandlung wurden entnommene Kannenmilchproben auf ihren Penicillingehalt kontrolliert und — in den Fällen mit Anzeigefarbe enthaltenden Versuchspräparaten — ebenfalls der Inhalt an solcher kontrolliert. Die Anzeigefarbe wurde im Laufe von zirka 2 Minuten mittels einer Ionenaustauschtechnik nachgewiesen, die bis zu 0,03 µg/ml herab empfindlich ist, was 0,002 I.E. Penicillin/ml entspricht.

Bei der Stichprobenkontrolle enthielten bis zu 3 % sämtlicher gelieferten Transportkannen (Tabelle 1) Penicillin (\geq 0,002 I.E./ml) und 7 von 574 Tankproben.

Gemäss der Tabelle 2 waren in der Zurückhalteperiode mehr als 10 % der Transportkannen mit Penicillin behaftet, falls bei der Mastitisbehandlung Handelspräparate und Versuchspräparate ohne Anzeigefarbe angewandt worden waren; dagegen erwiesen sich beim Gebrauch von Versuchspräparaten mit Zusatz von Food Green No. 4 weniger als 1 % kontaminiert.

Die Intensität und Verteilung der Penicillinkontaminierung variierte in den ersten 5 Tagen nach der Behandlung (Tabelle 3), indem die Kontaminierung bei den Handelspräparaten gegen Ende der Zurückhalteperiode am grössten war, während dieselbe betreffs der Versuchspräparate zu Beginn dieser Periode am stärksten war. Beim Gebrauch der Anzeigefarbe enthaltenden Versuchspräparate liess sich keine Regelmässigkeit in den Lieferungen penicillinkontaminierter Milch feststellen, und die gefundenen Konzentrationen waren wesentlich geringer als diejenigen Konzentrationen, welche nach Anwendung von Handelspräparaten und von Versuchspräparaten ohne Anzeigefarbe nachgewiesen wurden.

Die Anzeigefarbe schien keinen Einfluss auf den therapeutischen Effekt der Präparate ausgeübt zu haben.

Der obligatorische Zusatz von Anzeigefarbe zu Intramammaria dürfte eine schnelle Kontrolle der Milchlieferungen ermöglichen und ausserdem das Risiko einer unbeabsichtigten Beimischung penicillinhaltiger Milch einschränken.

RESUMÉ

Røbefarven Green S (Food Green No. 4) i penicillinholdige intramammaria.

III. Penicillinkontamineringen af leverandørmælk efter anvendelse af intramammaria med og uden røbefarve.

Der er i besætninger med akutte og kroniske mastiter behandlet, dels med almindelige i handelen værende mastitispræparater, dels med forsøgspræparater med eller uden røbefarve. Som forsøgspræparater er anvendt penicillin og penicillin-dihydrostreptomycin blanding i hydrofil vegetabilsk olie af typen viscoleo. Som røbefarve benyttedes Food Green No. 4 i en mængde af 50 mg. pr. 100.000 i.e. penicillin. Dosis af penicillin har pr. behandling varieret fra 100.000—300.000 i.e. pr. kirtel. Der er behandlet med handelspræparater i 14 besætninger, med forsøgspræparater i 19 besætninger og med forsøgspræparater tilsat røbefarve i 30 besætninger.

Lejlighedsvis udtoges spandemælksprøver fra samtlige leverandører og fra tankvogne med suppleringsmælk. Det første døgn efter behandlingen blev der udtaget spandemælksprøver, som kontrolleredes for indhold af penicillin og — i de tilfælde, hvor der anvendtes forsøgspræparater tilsat røbefarve — tillige for indhold af denne. Røbefarven påvist i løbet af ca. 2 min. ved en ionbytterteknik, der er følsom ned til 0,03 µg/ml., hvilket svarer til 0,002 i.e. penicillin/ml.

Ved stikprøvekontrollen fandtes penicillin ($\geq 0,002$ i.e./ml.) i op til 3 % af samtlige leverede transportspande (Tabel 1) og i 7 af 574 tankprøver.

Som det fremgår af tabel 2, fandtes i tilbageholdelsesperioden over 10 % af transportspandene kontaminerede med penicillin, når der ved mastitisbehandlingen var anvendt handelspræparater og forsøgspræparater uden røbefarve, mens mindre end 1 % fandtes kontaminerede, når der var anvendt forsøgspræparat med indhold af Food Green No. 4.

Penicillinkontamineringens intensitet og fordeling i de første 5 dage efter behandlingen (Tabel 3) varierede, idet kontamineringen for handelspræparaterne var størst hen imod slutningen af tilbageholdelsesperioden, mens den for forsøgspræparaterne var størst i tilbageholdelsesperiodens begyndelse. Ved anvendelse af de røbefarveholdige forsøgspræparater fandtes ingen regelmæssighed i leverancerne af penicillinkontamineret mælk, og de fundne koncentrationer var væsentlig mindre end de koncentrationer, der fandtes efter anvendelse af handelspræparater og forsøgspræparater uden røbefarve.

Røbefarven syntes ikke at have nogen indflydelse på præparaternes terapeutiske effekt.

Inkorporering af røbefarve i intramammaria vil muliggøre en hurtig kontrol med mælkeleverancerne og desuden mindske risikoen for utilsigtet tilblanding af penicillinholdig mælk.

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