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Residues of antibiotics in milk after intramammary application on high grade cows in Kenya (Zebu X Frisian and Zebu X Ayrshire)

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RÉSUMÉ

Résidus d'antibiotiques dans le lait après application intramammaire de vaches sélectionnées au Kenya (Zébu × Frisonne et Zébu × Ayrshire)

L'auteur étudie l'excrétion d'antibiotiques dans le lait après un traitement intramammaire appliqué à 26 vaches ayant un total de 54 quartiers malades. Il constate qu'un lait contenant plus de 0,05 UI d'un mélange de Pénicilline et Streptomycine par ml — ce qui est le cas jusqu'à 6 traites dans l'expérience — peut difficilement servir à la fabrication de produits laitiers fermentés.

I. INTRODUCTION

Public health authorities as well as dairy technologists are much concerned about the increasing use of antibiotics for therapeutic treatment of mastitis in milking herds. KREUZER (7) reviewed the mechanism of antibiotics excretion in milk and the public health importance of their presence in food. The quantity of antibiotics which is not absorbed by the udder tissues is excreted in the milk. Antibiotics in food can be dangerous for some human beings owing to their hypersensitivity to specific antibiotics. Furthermore, constant absorption of antibiotics diminishes their therapeutic value. The presence of antibiotics in milk disturbs all processing based on fermentation e. g. cheese, fermented milk like the « Mala » in Kenya (Maziwa lala) sour cream, butter. This means an economic loss for the farmer because the milk cannot be delivered to the dairy.

Unfortunately, the dairy industry has no economic way of eliminating these antibiotics. Even strong heat treatment has very little effect on their activities according to FEAGAN (3). There are some laboratory techniques using chemicals or enzymes e. g. Penicillinase, which can eliminate some specific antibiotic, but for economic reasons these methods are not applied on a commercial basis.

Tests on antibiotics residues in farm milk have been introduced in many countries. Severe fines strike the farmer who does not wait for the requested time before delivering the milk to the dairy (usually four days following treatment with antibiotics).

The question of antibiotics residues in milk and milk products as well as the methods of detection was studied by ALBRIGHT (1), FEAGAN (3), MARTH and ELLICKSON (9), MARTH (10), OVERBY (11), STORGARDS (14) and WITTER (15). HARGROVE *et al.* (4) mentioned an average Penicillin excretion of 37 p. 100 in the 1st milking and 26 to 49 p. 100 in all milkings. HORMAND *et al.* (5) found a Penicillin excretion of 21.4 p. 100 (2 to 84 p. 100) as an average for 100 treated cows. KOSIKOWSKI (6) reported that 2 cows out of 30 showed in the milk of untreated quarters, a Penicillin concen-

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tration of 0.03 IU/ml in the 2 milkings following treatment. COSGROVE and ETGEN (2) reported that 4 cows out of 33 had excretion of Penicillin in untreated quarters. HARGROVE *et al.* (4) reported an inhibition effect on *Streptococcus thermophilus* and *Lactobacillus bulgaricus* with a Penicillin concentration of over 0.01 IU and 0.1 IU/ml respectively. SHALO and HANSEN (13) observed that the growth of yoghourt and « Mala » cultures was clearly affected with a concentration of 0.16-1.6 IU Penicillin/ml. They recommend keeping the milk on the farm for 6 milkings. RICE (12) reported 0.03 IU Penicillin/ml as accepted limit for farm milk in Australia.

It was our intention to establish quantity and duration of antibiotic excretion in milk from treated and untreated quarters after intramammary application of antibiotics. We also wanted to obtain some knowledge on factors which affect the excretion of antibiotics.

II. MATERIAL AND METHODS

1. Sampling

The experiment was carried out in combination with a mastitis eradication program in a dairy herd at Lengenny farm, Kikuyu Division, Kenya. The cows were crossbreeds of Friesian, Ayrshire and Zebu breeds. Out of 59 milking cows, 26 had a total of 54 positive quarters in the California Mastitis Test, which represented 23 p. 100 of all quarters tested. Each positive quarter received an instillation of Vetramycin oily suspension consisting of 1.2 million units i. e. 600 000 IU Penicillin G Sodium and 600 000 IU Dihydro-Streptomycin Sulfate (Asid Bonz u. Sohn Gmbh, Lobhof-Munchen). Samples were taken from the milk of the treated quarters during the following eight hand milkings. Milking took place at 5 a. m. and 4 p. m.. Twenty two samples were also taken from the mixed milk of the untreated quarters. All milk samples were stored in the deep freezer at – 10 °C. Milk yields of the individual quarters were recorded as well.

2. Inhibition zones on agar plates, LEVETZOW (8)

a) Culture medium : Plate Count Agar (Oxoid CM 183).

b) The test organism : *B. subtilis*, was obtained from the German Public Health Administration. A lyophilised culture was distributed on the surface of the plate and incubated for 10 days at 30 °C. The spores were washed off the surface of the agar with sterile physiological NaCl solution and the suspension was centrifuged at 3 000 R/min. for 10 min. The sediment was resuspended in NaCl solution and centrifuged. The sediment was once more suspended in another sterile NaCl solution and heated at 70 °C for 30 min. Afterwards the suspension of spores was diluted to a concentration of 10^7 spores/ml (Plate Count method) and stored at 4 °C for a few months. No change could be observed during this time.

c) Preparation of plates : 0.5 ml of the prepared spores suspension was added to 500 ml plate count agar at 50 °C. After mixing, 15 ml of the liquid was poured onto each Petri dish (diameter 9 cm). The plates were then kept at 4 °C for further use (storing time was less than 3 weeks). One to 4 holes were cut in each plate.

d) Testing : the frozen milk samples were warmed up to 40 °C. Two drops of milk were placed in each hole and the plates were incubated at 30 °C for approximately 18 hours. Then, the radius of the inhibition zone (radius of the hole excluded) was measured and converted into IU/ml according to figure 1. The total excretion of antibiotics was calculated from the milk yield (table I).

e) Standard curve : 3 sets of standard solutions of Vetramycin (0.001-5 000 units) were prepared by diluting Vetramycin with raw and UHT milk and tested as fresh samples, as well as after deep freezing (fig. 1).

Fig. 1-Standard curve for Vetramycin.

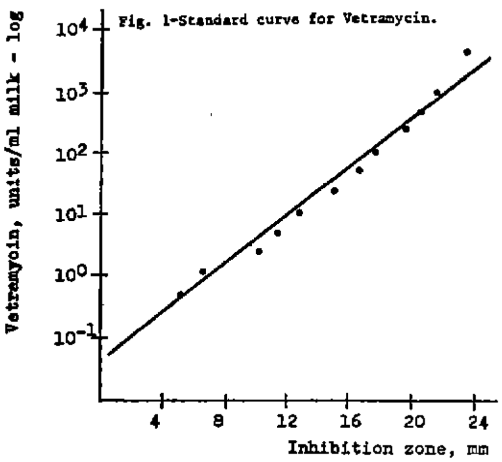

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Table I. Vetramycin excretion in milk from 54 quarters : average per quarter (Inhibition zone test).

Number of milking	Number positive qtr	milk yield in ml	Units/ml	Total units/qtr*	$\frac{\text{units/qtr}}{1,200,000}$ x100
1	54	756	313	236,628	19.7
2	54	725	31	22,475	1.9
3	52	773	3.2	2,474	0.2
4	49	717	0,52	373	0.03
5	29	714	0.112	80	0.007
6	10	625	0.024	15	0.001
1-6		4310		262,045	21.8

* Fig. 2

3. Acid production

The standard solutions prepared for the inhibition zone test were also used for the acid production test. As this test takes more time but is slightly more sensitive, only samples with negative results in the inhibition zone test and samples from the untreated quarters were considered (table III). The acidity test was performed as follows : 0.6 ml of a yoghourt starter culture (*Str. thermophilus* and *Lb. bulgaricus*) was added to 20 ml milk. The pH was measured after 3 hrs of incubation at 42 °C (table II and III).

Table II. Inhibition zone and acid production (Mean values of 3 sets of standard solutions).

Vetramycin concentration units/ml	Inhibition zone (*B.subtilis*) (mm)	pH after 3 hours (yoghurt-starter)
5,000	23.5	6.45
1,000	21.5	6.45
500	20.5	6.45
250	19.3	6.45
100	17.5	6.40
50	16.5	6.40
25	14.8	6.30
10	12.7	6.15
5	11.3	6.10
2.5	9.7	6.10
1	6.6	5.85
0.5	5.2	5.30
0.1	0	5.10
0.05	0	4.60
0.01	0	4.60
0.005	0	4.60
0.001	0	4.60
0 (control)	0	4.60

4. Factors affecting the antibiotics excretion

Amount and type of antibiotics, solvent base, combination with other pharmaceutical products, way of instillating the tube in the teat, yield, cell count etc, can affect the quantity of antibiotics excreted in the milk. Of these factors we examined the milk yield (fig. 3) and the cell count (fig. 4). The cell count was determined by the coulter count method.

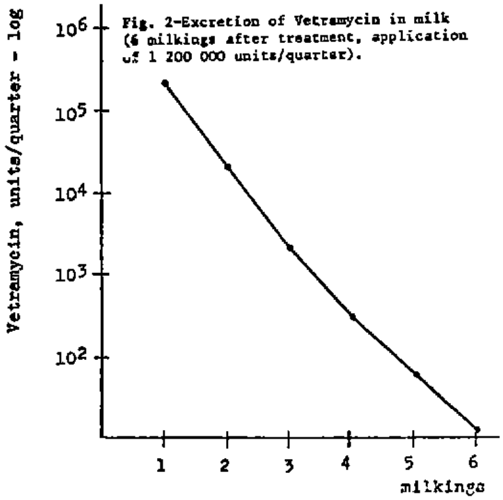

Fig. 2-Excretion of Vetramycin in milk (6 milkings after treatment, application of 1 200 000 units/quarter).

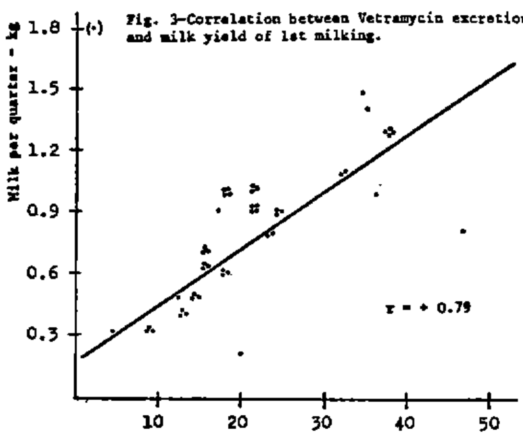

Fig. 3-Correlation between Vetramycin excretion and milk yield of 1st milking.

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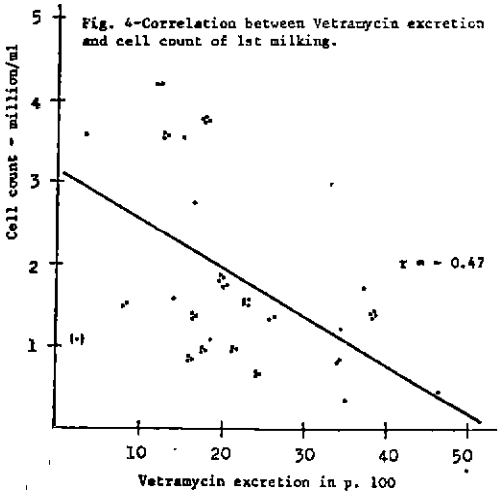

Fig. 4-Correlation between Vetramycin excretion and cell count of 1st milking.

III. RESULTS

Inhibition zone and acid production were identical for fresh and frozen samples. To check the reproducibility of the *B. subtilis* test, 41 parallel tests were carried out with milk containing 15 units of Vetramycin per ml. The average inhibition zone was 13 mm with variations between 12 and 14 mm.

From the total instilled Vetramycin of 1 200 000 units, excreted quantities varied between 2.5 and 46.9 p. 100 with a mean of 21.8 and a standard deviation of ± 8.8 p. 100. At the 3rd milking after treatment, the milk of two treated quarters was free of antibiotic. No residues could be traced at the 7th milking.

All the negative results of the inhibition zone test were checked and confirmed to be negative with the acidity test.

Table III Acidity test and calculated Vetramycin content of the milk from untreated quarters of 22 cows (3 milkings)

cow nr.	pH after 3 hours milking		Units/ml milk milking	
	1	2	1	2
1	5.9	4.6	1	0
2	5.6	5.15	0.7	0.2
3	5.6	4.6	0.7	0
4	4.9	5.7	0.1	0.8
5	4.6	5.5	0	0.6
6-22	4.6	4.6	0	0
Average			0.1	0.07
Control	4.6	4.6	0	0

All samples were negative at the 3rd milking

IV. DISCUSSION AND CONCLUSIONS

a) *Sensitivity of the test*

The inhibition zone test can be used for the detection of a minimum concentration of 0.5 units Vetramycin/ml.

The acidity test shows a minimum concentration of 0.1 units/ml. It can therefore be recommended for the control of bulk milk in the factory before processing.

b) *Excretion of Vetramycin from treated quarters*

An average of 21.8 p. 100 (± 8.8 p. 100) is excreted with the milk : 19.7 p. 100 in the first milking and 1.9 p. 100 in the 2nd milking. The regression proved to be logarithmic. Traces could still be found in the 6th milking.

c) *Use of milk containing antibiotics*

When the milk of one treated quarter is mixed with the milk of untreated quarters or healthy cows, the concentration must not exceed 0.05 units/ml (table 1). Our results proved that with this concentration it is still possible to manufacture milk products. For the 1st milking, an average of 6 000 untreated quarters is necessary to bring down the concentration from about 300 to 0.05 units/ml. The milk from treated quarters should therefore be fed to the farm animals.

d) *Excretion of Vetramycin from untreated quarters*

In the milk of 5 cows out of 22, traces of Vetramycin were found in the 1st and 2nd milkings. An average concentration of 0.1 units/ml will almost not affect the processing of any dairy products because the dilution with the milk from healthy cows eliminates the inhibitory effect. For practical use, however, only milk from untreated cows should be accepted.

e) *Correlation of Vetramycin excretion with milk yield and cell count*

Figure 3 shows a distinct correlation between milk yield and Vetramycin excretion (r = + 0.79). Further studies might give an explanation for these interesting facts.

Figure 4 shows another, however not so distinct, correlation between cell count and Vetramycin excretion (r = − 0.47).

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SUMMARY

Residues of antibiotics in milk after intramammary application

26 cows (54 quarters) were treated for mastitis with intramammary application of 1 200 000 units of Vetramycin (600 000 IU Penicillin + 600 000 IU Dihydro-Streptomycin). Samples were taken from the eight consecutive milkings after treatment. A total of 21.8 p. 100 (± 8.8 p. 100) of the instilled Vetramycin was released in the milk from treated quarters. This excretion was mostly in the 1st and 2nd milkings which had 19.7 and 1.9 p. 100 respectively. Correlations were established between excreted Vetramycin and milk yield (r = + 0.79) as well as cell count (r = — 0.47).

RESUMEN

Residuos de antibioticos en la leche después de aplicación intramamaria en vacas (Cebú × Frison y Cebú × Ayrshire) en Kenia

El autor estudia la excreción de antibioticos en la leche después de un tratamiento intramamario aplicado en 26 vacas teniendo un total de 54 cuartos enfermos.

Comproba que una leche conteniendo más de 0.05 UI de una mezcla de penicilina y estreptomicina por ml — lo que es el caso hasta 6 ordeños en esta experiencia — puede dificilmente servir para la fabricación de productos lecheros fermentados.

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