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Occurrence of Chlorfenvinphos Residues in Cow's Milk Sampled at a Range of Sites in Western Kenya

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Kenya's fast growing human population is expected to reach 35 million by the year 2000. In order to cope with such a rapid rate of growth, efforts must be directed towards adequate agricultural and livestock production to counter the disproportionate increase in demand for food. To provide sufficient animal protein (milk and beef products) attempts must be made to eliminate the current constraints hindering livestock production and expansion in Kenya (KARI,1989). One such constraint (in terms of both health effects and economic losses) is the presence of several important infectious diseases affecting cattle, characterized by the occurrence of parasites in the animal's blood (haemoparasites) (Mutugi *et al*, 1989).

There are two major groups of haemoparasitic diseases that occur in Kenya: tick-transmitted, and tsetse and non-tsetse transmitted (trypanosomiasis) diseases. Tick-borne diseases are considered to be the most important animal health problem in the high potential areas, while trypanosomiasis is a major threat in the low potential range lands (Mutugi, 1986). These diseases restrict introduction of higher producing but susceptible stock in certain areas of the country; inflict high mortalities in susceptible stock; lead to productivity losses in recovered animals; and necessitate exclusion of highly productive breeds of livestock from locations where there is an outbreak (FAO, 1984).

Tick-borne diseases frequently encountered in Kenya are *theileriosis, anaplasmosis, cowdriosis* and *babesiosis. Theileriosis* comprises a group of protozoan parasites of the genus *Theileria,* which are transmitted by the ixodid ticks. Four different species of this genus are recorded in cattle; clinical theileriosis is associated with one species, *Theiletia parva* transmitted by the brown ear tick, *Rhipicephalus appendiculatus.* This species causes the notorious East Coast Fever (ECF), a highly fatal disease of cattle. A closely related form, corridor disease *(T.parva Tawrencei* infection) transmitted by the same tick is a buffalo derived parasite that causes very high mortalities in infected cattle (Mutugi *et al,* 1989). In Western Kenya, both ECF and anaplasmosis are common practical animal health problems that are seriously affecting the livestock industry. Outbreaks of these diseases are frequent and have continued to pose great challenges in terms of control for over 80 years.

Currently, the most conventional method of controlling ECF and anaplasmosis in cattle

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involves the use of acaricides. In Western Kenya, many types of acaricides are available but presently, the most commonly used chemical is chlorfenvinphos (ILRAD, 1991). It is frequently applied on cattle either through plunge dips or sprays. Little, however, is known about the fate of this compound and its residual effect in milk and beef. A recent survey in Kenya (KEMRI,1988) suggests that chronic or acute exposure to chlorfenvinphos can result in serious health effects in humans. Residue levels exceeding 8µg/kg of butterfat in cow's milk are currently regarded as dangerous for human consumption (Codex Alimentarius,l993), although concentrations as high as 2Oµg/kg have been reported in Australia (Shell, 1969).

The purpose of this project was to establish the levels of chlorfenvinphos typically occurring in Kenyan cow's milk; and to determine the influence of season (climate changes) variation in butterfat content, and method of acaricide application (plunge dip or spray) on the residue content in milk sampled at a range of sites in Western Kenya.

MATERIALS AND METHODS

Chlorfenvinphos standard of 1.361 g/cc density and 99.5% purity was supplied by Kenya-Swiss Chemical Company (Ciba-Basel). HPLC grade acetonitrile and methanol and analytical grade acetone and Florisil gel were purchased from Sigma Chemical Company, UK. Reagent-grade n-hexane, dichloromethane, anhydrous sodium sulphate and phosphate buffer (pH 6.0) were obtained from international suppliers in Nairobi. The hexane and dichloromethane as well as deionized water were distilled twice before use.

A Perkin-Elmer Model 8500 Gas chromatograph fitted with a flame ionization detector was used for chromatographic analyses along with a capillary SE-45 column under isothermal conditions, while a VWR Scientific pH meter was employed for all pH determinations. A Beckmann Model 334 Gradient Liquid Chromatograph equiped with a Hitachi 100-40 UV detector and fitted with µBondapak C18 column was used for all HPLC analyses. A GC-Mass Spectrometer Model VG 12-250 (courtesy of the International Center for Insect Physiology and Ecology, Nairobi) fitted with a capillary Ultra 1 column was used in all confirmatory analyses. All runs were programmed for 52 minutes between 50 C and 280 C. An IEC Clinical Centrifuge Model CAT 801 was employed for all centrifugations.

One hundred milk samples representing a variety of farms in Bungoma and Trans Nzoia districts of Western Kenya, were collected during both the dry (December-March) and wet (April-August) seasons in 1993 and 1994. Samples (each about 300 ml) were stored in sterilized plastic bottles at -50 C prior to laboratory analysis.

Samples were extracted according to the procedure reported by McLeod and Ritcey(1973). An aliquot (100g) was transferred quantitatively into a Waring commercial blender and 90ml of acetone and 50ml hexane added. The mixture was blended for three minutes to give a slurry which was then centrifuged at 537g for 5 minutes. The yellow hexane layer containing butterfat was transferred into a clean beaker using a teat pipette, and dried with 5g of anhydrous sodium sulphate for ten minutes. This extract was then filtered through Whatman No. 1 filter paper and the solvent removed using a rotary evaporator (Achelis & Sons, Germany). The oil was then transferred quantitatively to a pre-weighed vial using a minimum of

dichloromethane which was thereafter expelled on a waterbath at 40 C. The weight of the dry, solvent-free butter-oil residue was recorded.

The butter-oil extract was redissolved in hexane (40ml) and shaken with four 5-ml portions of acetonitrile in a separatory funnel. To the combined acetonitrile phase was added 5ml dichloromethane, 5ml phosphate buffer, 50ml of hexane, l00ml distilled-deionozed water, and 15ml of saturated sodium sulphate solution. The mixture was then vigorously shaken for 2 minutes and allowed to settle. The hexane phase was washed twice with 5ml portions of water, and then filtered through Whatman No.1 filter paper containing 25g of anhydrous sodium sulphate. The filtrate was evaporated to about 5ml and quantitatively transferred to a chromatographic glass column (30cm x 22mm i.d.) packed with 9g Florisil in hexane. The extract was eluted first with 25ml of hexane followed by 50ml of a mixture of hexane and dichloromethane (1:1), and finally 25ml of dichloromethane. The eluent portions were combined and evaporated to about 5ml on a rotary evaporator. The final residue was made to 10m1 with dichloromethane, prior to chromatographic analysis.

For HPLC analysis, investigations revealed that aqueous methanol (70% v/v) was the most suitable solvent system. Analysis was performed at a fixed attenuation setting using ambient conditions. Measurements made at 254nm using a flow rate of 2ml/min gave effective separation of peaks and retention times of 1.8 minutes for chlorfenvinphos. Acceptable recoveries ranging from 89-106% (Table 1) were also obtained.

Some of the milk samples that showed presence of chlorfenvinphos residues were further subjected to comparative analysis using GC (Retention time 35.22 minutes) and MS detection peaks at m/e= 81, 109, 279, 295 and 323. This was done according to the procedure of Gilbert *et al* (1987) with Helium as carrier gas. Data was tested for significant difference using conventional students t-test and one-way analysis of variance.

Table 1. Recoveries of chlorfenvinphos and 2,4-dichloroacetophenone from spiked cow milk.

CHLORFENVINPHOS		2,4-DICHLOROACETOPHENONE	
Amount spiked (ng)	% Recovered	Amount spiked (µg)	% Recovered
1.0	89.0	1.0	92.4
0.9	94.7	0.9	94.6
0.8	93.2	0.8	100.3
0.7	90.2	0.7	111.1
0.5	95.9	0.6	93.4
0.2	93.2	0.4	86.4
0.1	93.3	0.3	93.2
0.075	106.6	0.075	87.9

RESULTS AND DISCUSSION

Tables 2 and 3 give data on the residues of chlorfenvinphos in milk for the two seasons.

Table 2. Chlorfenvinphos residues (µg/kg) in milk from Bungoma district.

CENTER	WET SEASON		DRY SEASON	
	Mean (±sd)	Median	Mean (±sd)	Median
Sang'alo Inst.	2.88 (0.33)	2.7	1.46 (0.95)	1.17
LBDA	4.53 -	-	1.28 (1.07)	1.28
F T C	2.79 (0.14)	2.79	1.18 (0.12)	1.16
Kitinda Center	3.62 (0.74)	3.92	1.84 (0.66)	1.84
Ndalu Center	6.75 (0.76)	7.08	2.18 (1.44)	2.18
Khakula Farm	6.03 (4.58)	6.03	1.44 -	-
Okwomi Farm	5.14 (3.49)	5.14	N.D. -	-
Barasa Farm	N.D.	-	N.D.	-
Kituyi Farm	2.04 -	-	3.9 -	-
Okonya Farm	2.45 (1.13)	2.45	-	-

N.D. = Not detected

Table 3. Chlorfenvinphos residues µg/kg) in milk from Trans Nzoia district.

CENTER	WET SEASON		DRY SEASON	
	Mean (±sd)	Median	Mean (±sd)	Median
Cherangani Center	3.86 (0.84)	3.86	1.97 (1.21)	1.32
Nzoia Scheme	3.00 (0.3)	3.00	2.20 -	-
Wambwa Farm	1.85 -	-	2.26 (0.61)	2.26
Kihara Farm	2.79 -	-	1.30 (0.21)	1.30
ADC Farm	3.27 -	-	-	
Soy Sambu Center	3.67 (1.33)	3.9	-	
Bikeke Center	4.61 (3.01)	3.09	-	
Kapsara Center	3.05 (2.05)	2.19	-	
Sikhendu Center	10.40 (0.83)	10.40	-	

Milk samples collected in the wet season had higher ($P<0.05$) values of the residue (range 1.85-10.4 µg/kg) than those taken during the dry season (range 1.18-3.9 µg/kg).

Only at one sampling point (Sikhendu) did the residue content exceed the Codex (1993) critical limit of 8 µg/kg milkfat . Majority of the levels remained below this recommended limit during the rainy season and were extremely low during the dry season. The low levels detected during this period were probably due to other secretion routes mainly via the gastro-intestinal ingestion or through lungs by inhalation.

Rainy conditions, and the characteristics of additives used in the commercial formulations probably promote prolonged retention of the acaricide on the animals'

skin. For example, because of the high boiling point and lipophorbic nature of water relative to the active ingredient, aqueous environments tend to provide an extra layer over the acaricide on the animal's wet coat, thus creating prolonged dermal exposure periods. Increased absorption is hence expected. Acaricide drip-off during the milking process is high during the wet season and can contaminate the milk. These factors may therefore help to explain why the residue contents were generally higher in the wet than the dry season irrespective of the butterfat content. Table 4 gives the data on chlorfenvinphos residues and milkfat content for the wet season.

Table 4. Percent milkfat and mean levels of chlorfenvinphos (µg/kg) residues in wet season samples.

CENTER	No. of Samples	Mean % milkfat	Chlorfenvinphos Residues		
			Mean(±sd)	Median	Range
Khakula F a r m	2	5.13	6.03(4.58)	6.03	2.79-9.27
Okwomi Farm	2	4.06	5.14(3.49)	5.14	2.67-7.60
LBDA	4	4.60	4.53 -	-	-
Cherangani Cen.	3	4.03	3.86(0.84)	3.86	3.32-4.41
Nzoia Scheme	2	3.69	3.00(0.3)	3.00	2.79-3.21
Sang'alo Inst.	4	3.68	2.88(0.33)	2.70	2.66-3.29
F T C	4	3.09	2.79(0.14)	2.79	2.69-2.89
Okonya Farm	2	2.91	2.45(1.13)	2.45	1.65-3.25
Kituyi Farm	2	2.15	2.04 -	-	-
TOTALS	25	3.70	3.64(0.83)	4.81	1.65-9.27

Correlation of the milkfat data with the concentrations of chlorfenvinphos residues gave a coefficient of($r=0.8225$)which was significant ($P<0.05$), suggesting a linear relationship between the two quantities for samples collected in the wet season (Fig. 1).

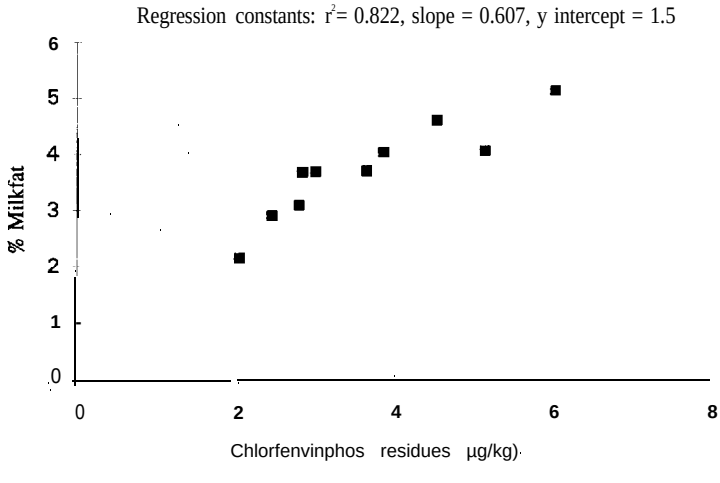

Figure 1. Relationship between percent milkfat and chlorfenvinphos concentration in wet season.

By contrast, there was no such relationship between the acaricide residues and milkfat content for samples collected during the dry season (r=-9.98). Tables 5 and 6 provide data for residues of chlorfenvinphos in milk samples from dipped and handsprayed cows, respectively.

Table 5. Mean (±Standard Deviation) chlorfenvinphos residues samples obtained from dipped cows.

CENTER	CHLORFENVINPHOS RESIDUES µg/kg)			
	WET SEASON		DRY SEASON	
	Mean (±sd)	Range	Mean (±sd)	Range
Sang'alo Inst.	2.88 (0.33)	2.66- 3.29	1.46 (0.95)	0.69- 1.17
LBDA	4.53 -	-	1.28 (1.07)	0.52- 2.04
F T C	2.79 (0.14)	2.69- 2.89	1.18 (0.12)	1.05- 1.33
ADC Farm	3.27 -	-	-	-
Sikhendu Center	10.40 (0.83)	9.78- 10.96	-	-
Ndalu Center	6.75 (0.76)	5.86- 7.3	2.14 (1.44)	1.12- 3.16
Bikeke Center	4.61 (3.01)	2.67- 8.07	-	-
Kitinda Center	3.57 (0.74)	2.97- 3.98	1.84 (0.66)	1.37- 2.31
Cherangani Cen.	3.86 (0.84)	3.22- 4.41	1.97 (1.21)	1.22- 3.36

Table 6. Mean (5 Standard Deviation) chlorfenvinphos residues in samples obtained from handsprayed cows.

CENTER	CHLORFENVINPHOS RESIDUES µg/kg)			
	WET SEASON		DRY SEASON	
	Mean (±sd)	Range	Mean (±sd)	Range
Wambwa Farm	1.85 -	-	2.26 (0.61)	1.83- 2.69
Khakula Farm	6.03 (4.58)	2.79- 9.27	1.44 -	-
Okwomi Farm	5.14 (3.49)	2.69- 7.60	N.D.	-
Kituyi Farm	2.04 -	-	3.90 -	-
Kihara Farm	2.79 -	-	1.3 (0.21)	1.15- 1.45
Okonya Farm	2.45 (1.13)	1.68- 3.25	-	-
Barasa Farm	N.D.		N.D.	

N.D. = Not detected

It was observed that the residues were generally higher in milk sampled from animals that were dipped than in those that were handsprayed ($P<0.05$). The higher degree of ingestion of the acaricide by animals during the dipping operation and the prolonged dermal exposure period due to the skin contact with mud and dung that were present in the public plunge dips are suggested as the probable causes.

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