SCREENING OF CATTLE BLOOD SAMPLES FROM KASARGOD DISTRICT FOR PRESENCE OF HCH AND DDT RESIDUES

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Abstract

A study was undertaken to investigate presence of HCH and DDT residues in bovine blood samples of Enmakaje panchayat of Kasargod district. Retrospective analysis of case sheets for last three years at the local veterinary dispensary was also carried out. Whole blood samples were collected from 20 randomly selected cows for pesticide residue and haematological analysis. Collected samples were processed by various clean up procedures and analysed using Gas Chromatography for HCH and DDT residues. Residues of DDE and DDT were below detection limit (BDL) in all the analysed serum samples. The haematological values of cattle from study area remained within the normal range except differential leucocyte count which exhibited marked lymphocytopenia. In the present study, the level of pesticide residues in the serum samples in the study area was not enough to produce any visible health hazards in cattle.

Key words: *HCH, DDT residues, Cattle blood, Gas Chromatography*

Organochlorine pesticides (OCPs) such as hexachlorocyclohexane (HCH) and dichlorodiphenyltrichloroethane (DDT) are persistent, broad-spectrum toxicants. They resist biodegradation and therefore can concentrate through food chains to produce a significant magnification of the original concentration in the food web with high risks to the ecosystem and human health (Leena *et al.*, 2011).

Enmakaje panchayath of Kasargod district has long been in the media due to its burden of illness generated by intensive and broad usage of Endosulfan by Plantation Corporation of Kerala. Research of past years has identified potential entry routes of pesticides into the environment such as spray drift, volatilisation and precipitation, surface runoff/overland flow, leaching, drainflow and base flow seepage (Carter, 2000). There has been reports of birth of calves with deformed limbs in the animal population of this area. Therefore this study was structured to investigate for the presence of HCH and DDT residues in cattle blood and also to study the haematological pattern of the same animals.

Materials and Methods

Padre, Perla, Swarga and Vani nagar villages of Enmakaje Panchayath of Kasargod district were selected as study area.

A preliminary survey was conducted among 60 farmers belonging to the Panchayath for identifying the disease pattern in cattle. For this a proforma was designed to assess the disease conditions of cattle, rearing system, the clinical course of illness, treatment adopted and disease outcome and then

distributed among the farmers. The responses indicated that conditions as infertility, cancer, congenital anomalies, repeated abortions were frequent in animals as in humans. The sampling locations were identified and fixed after consulting veterinary surgeon, panchayath officials and health officials of this area. Further, a retrospective analysis of cases presented at the Badiadka Veterinary dispensary in the past three years was done to ascertain the disease pattern and trends prevailing in the area.

Blood samples were collected from randomly selected 20 adult cattle of the area which were maintained for a period of three years or more. Further, blood samples were collected from 20 healthy cattle in the University Livestock Farm (ULF), Mannuthy and these were used as control for haematological analysis. Samples collected were immediately placed in cool box maintained at 4-8p C and transported in similar condition to the laboratory and stored at -20p C until analyzed.

Pesticide residue extraction by Pitarch *et al.* (2005) was followed. The serum separated from blood was stored in clean dry glass vials with stopper. From this, 2 ml serum was taken in a 20 ml separating funnel. It was

Fig.1. Chromatogram of HCH and DDT Standard and their retention time

Fig.2. Chromatogram of Blood sample

diluted with 3 ml deionised water. To this added 5 ml dichloromethane and shaken well for five minutes. Repeated this procedure and extract was centrifuged to remove the emulsion obtained. The organic layer formed after extraction was then separated and dried by passing through a column packed with 3 cm layers of anhydrous sodium sulphate. The extract was evaporated to dryness under a gentle stream of air and residue was dissolved in 1ml of n-hexane for injection into the Gas Chromatography (GC).

Hexachlorocyclohexane and dichlorodiphenyltrichloroethane content in serum samples were quantified by GC. Analysis was done in SHIMADZU-2010 GC with electron capture detector (ECD) having [63]Ni as radioactive source.

Haematological parameters were studied using blood samples with potassium EDTA added as anticoagulant. Total erythrocyte count (TEC), haemoglobin (Hb), volume of packed red cells (VPRC), total leucocyte count (TLC), differential leucocyte count (DLC) and Erythrocyte indices like Mean corpuscular volume (MCV), Mean Corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) were also calculated using techniques given by Benjamin, 1985.

Statistical comparison of results from exposed and non-exposed animals was performed using the *student t-test* by using Statistical Package for the Social Sciences (SPSS) 17 version.

Results and Discussion

A total of 8843 cases had been recorded at the Badiadka Veterinary dispensary in the following pattern of decreasing occurrence: digestive disorders (53.4%), parasitic diseases (13.2%), reproductive diseases (10.48%), respiratory disease (6.5%), skin disorders (6.22%), metabolic disorders (3.7%), others (3.5%), fever (2.8%) and poisoning (0.2%).

Analytical method for estimation of HCH and DDT

The chromatogram of mixed standards depicted each isomer of HCH (alpha, beta, gamma and delta) and DDT (*p'p*-DDE, *p'p*-DDD and *p'p*-DDT) as a separate and well defined peak (Fig.1). Under the identical operating condition of GC, the
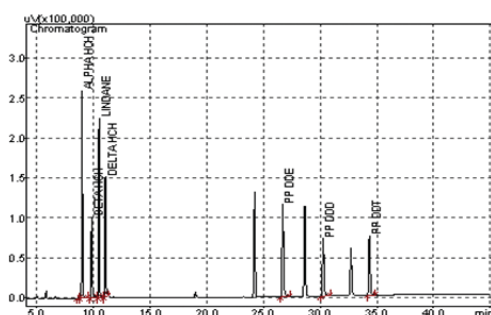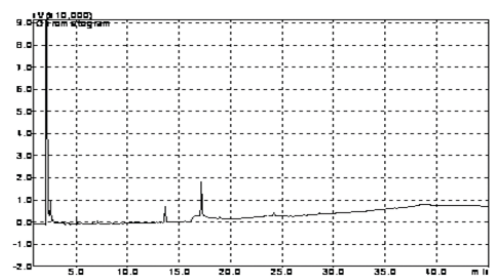
retention time (RT) for each component was 8.981, 9.812, 10.466, 11.017, 26.691, 30.256 and 34.339 minutes respectively. Recovery studies revealed the percentage of recovery of HCH and DDT to be about 70-80 per cent which is satisfactory to carry out sample analysis. HCH and DDT residues were below detection limit in all of the blood samples collected from study area. The chromatogram of a blood samples collected from study area is shown in Fig.2. The human and animals pesticide residue in serum is a biological index of pesticide exposure and studies on blood can be used for assessing the total body burden of pesticides in the general population (Saxena *et al.,* 1987). Mean value of total HCH and DDT in serum samples of cattle from the study area were below the detection level. Kaphalia and Seth (1984) detected total HCH level in buffalo and goat serum samples were below the generally accepted safe level of 50ppb. Blood sampling is considered as a valid method for measuring body burden of persistent organochlorine contaminants (Dhananjayan and Muralidharan, 2010). Present study revealed that, HCH and DDT were not present at a detectable level in serum samples.

Haematological Parameters

Total erythrocyte count (TEC), haemoglobin (Hb), volume of packed red cells (VPRC), total leucocyte count (TLC), differential leucocyte count (DLC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were estimated and the values are presented in Table.1.

Mean RBC count (millions/µl) of the samples from Kasargod and samples from University Livestock Farm (ULF), Mannuthy were 5.721±0.29835 and 5.2740±0.20974 respectively. The values were in accordance with the normal erythrocyte count in cattle of 5 to 10 millions/ µl (Kaneko *et al.*, 1997).

The mean WBC count (number/ µl) of the cattle from study area was 9918±919.443 and that of the ULF, Mannuthy was 8528±407.439. Thus, the TLC in the cattle of study area was more than that of control group. But, the values lie within the normal range of 4000 to 12,000 numbers/ µl (Kaneko *et al.*, 1997).

Volume of packed red cells (%) of blood samples from Kasargod and ULF, Mannuthy area were 26.06±2.08967 and 24.83±1.01686 respectively. No significant statistical difference was observed between the two groups. The samples from study area exhibited VPRC values in the normal range of 24 to 48 per cent (Benjamin, 1985).

Average Hb concentration (g %) of samples from Kasargod and samples from ULF, Mannuthy were 9.67±0.41313 and 8.38±0.32857 respectively. The values from cattle of both areas found to be within the normal range of 8 to 15g per cent (Benjamin, 1985).

The mean neutrophil percentage in blood samples from Kasargod area and ULF Mannuthy samples were 25.3±2.70 and 18.60±2.77 per cent respectively. No significant difference existed between the two groups (Table 1). The mean lymphocyte percentage in blood samples from study area (59±4.03 per cent) was significantly (P<0.05) lower than the samples of ULF, Mannuthy (76.8±3.60 per cent). Mean eosinophil percentage of blood samples from study area and Mannuthy area were 14.80±3.54 per cent and 12.70±7.64206 per cent respectively. No significant statistical difference was observed between the two groups. The mean monocyte count in blood samples of study area and ULF, Mannuthy samples were 3.55±0.50 per cent and 2.2±0.554 per cent respectively. No significant difference existed between the two groups.

Mean corpuscular volume (MCV) of cattle from Kasargod and ULF, Mannuthy area were 49.41±1.67 and 47.37±1.78 (fl) respectively. There was no significant difference between the two groups (Table 1). The mean corpuscular haemoglobin (MCH) values of cattle from study area and control samples were 17.00±0.41 and 15.97±0.47 (pg) respectively. No significant difference was observed between the values (Table 1). Mean corpuscular haemoglobin (MCHC) value of cattle from Kasargod and ULF, Mannuthy were 34.53±0.38 and 33.79±0.35 (g%) respectively. Statistical analysis showed that there was no significant difference observed between the two groups (Table).

The present study revealed that the HCH and DDT residues in the blood of cattle from Enmakaje panchayath of Kasargod district are below the detectable level and the haematological picture showed that these animals are healthy.

Acknowledgement

Table. Haematological parameters of cattle in Kasargod and ULF, Mannuthy

Sl. No.	Parameters	Kasargod	ULF, Mannuthy
1	RBC (Millions/µl)	5.72 ± 0.29^a	5.27 ± 0.20^a
2	WBC (number/ µl)	9918 ± 919.44^a	8528 ± 407.43^a
3	Volume of Packed Red Cells (VPRC) (%)	26.06 ± 2.08^a	24.83 ± 1.016^a
4	Hb (g/dl)	9.67 ± 0.41^a	8.38 ± 0.32^a
5	Differential leucocyte count (%)		
	Lymphocytes	59 ± 4.03^a	76.8 ± 3.60^a
	Neutrophils	25.30 ± 2.70^a	18.60 ± 2.77^a
	Eosinophils	14.80 ± 3.54^a	12.70 ± 7.64^a
	Monocytes	3.55 ± 0.50^a	2.2 ± 0.554^a
	Basophils	-	-
6	MCV (fl)	49.41 ± 1.67^a	47.37 ± 1.78^a
7	MCH (pg)	17.00 ± 0.41^a	15.97 ± 0.47^a
8	MCHC (g%)	34.53 ± 0.38^a	33.79 ± 0.35^a

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