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Organochlorine, organophosphate, synthetic pyrethroids assessment in bovine milk and water samples of Jaipur, Rajasthan.

S SHARMA[1], V SHARMA[2], J JAIN[3], S AGARWAL[4], D K DAHIYA[5] and A KHAN[6]

Rajasthan University of Veterinary and Animal Sciences, Jaipur, Rajasthan 302 020 India

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ABSTRACT

Important pesticides (organo- chlorine, -phosphate, and synthetic pyrethroids) in bovine milk samples, water and fodder collected from the eight different blocks of Jaipur, Rajasthan were monitored in the present study. Simultaneously, a survey was conducted regarding the usage of pesticides in these areas in particularly on animal feed. Sixty four samples each of wheat (*Triticum*) straw and *bajra* (*Pennisetum glaucum*) stover, 32 samples each of lucerne (*Medicago sativa*), water, indigenous cow milk, 64 samples each of exotic cow and buffalo milk were analysed. Gas chromatography results revealed that pesticides in milk, water and fodder samples were absent. The attributed reason being the low consumption and prevailing environmental conditions, viz. soil type, surface water depth, intensity and length of sunlight exposure, temperature etc. which leads to the faster degradation of these pesticides.

Key words: Bovine milk, Fodder, Gas-chromatography, Pesticides, Rajasthan, Water

Milk is a nutritious food for all age groups, which might get contaminated by various adulterants and pesticides, during processing and improper handling (Abou *et al*. 2010). Indiscriminate use of these pesticides has increased their level in the food chain that has led to the deleterious health consequences on the consumer (Jaraczewska *et al*. 2006). Majority of organochlorine pesticides are banned in India but still some of them are used in agriculture and malaria control programme. These pesticides are not easily bio-degradable and therefore persist in the environment for longer period (Rajendran 2003). Earlier many Indian studies claimed contamination of the milk samples collected from different regions of India (Pandit *et al*. 2002, Kaushik *et al*. 2011, John 2001).

By visualizing the scarcity and associated production cost of animal feed and fodder in the state farmers are forced to depend on other forage sources like spoiled vegetables, fruits and other crops. These crops are often sprayed with various pesticides and hence can make their route into the animal body by feeding. Many investigators detected considerable presence of pesticides in other daily ingested foods. Moreover, district Jaipur is counted among the highest milk producing regions of Rajasthan. So, the milk contamination in this region is the major issue of public concern. Therefore the study was designed to assess the preliminary status regarding the presence of pesticides in milk samples collected from Jaipur district.

Present address: [2]Professor and Head (drvishnus @yahoo.com), Post Graduate Institute Veterinary Education and Research, B-2 By-pass, Shipra path, Agarwal farm.

MATERIALS AND METHODS

Collection of milk, water and fodder samples: On the basis of highest number of milch animals of the area, district Jaipur was categorized into 8 blocks as; Amer, Kotputli, Shahpura, Chomu, Chaksu, Phulera, Jamvaramgarh and Bassi for collection of milk samples. A questionnaire was prepared and people employed in milk production were asked to fill up the performa regarding the use of pesticides in the area, particularly on animal feeds, vegetable crops and various other crops. For the estimation of pesticides, milk samples were directly obtained from the farmers to rule out the possibility of extrinsic contamination during collection and transportation. Total 160 milk samples (32 cow indigenous, 64 cow exotic, 64 buffalo), 160 fodder samples (64 each of wheat straw and *bajra* strover, 32 lucern), 32 water samples were collected and analyzed for the different pesticide residues including organochlorines, organophosphates and synthetic pyrethroids. All samples were stored at 4°C until analyzed.

Chemical and reagents: The gas chromatography grade (~99%) standards; organochlorine (aldrin, DDT p, p'-DDT), endosulfan- *alpha* and *beta*, HCH isomers-*alpha, beta, gamma* and *delta*, heptachlor) and organophospates (dichlrovos, dimethoate, methylparathion, malathion, chlorpyrifos, quinalfos, profenofos, ethion and phosalone) and synthetic pyrethroids (fenpropathrin, cyhalothrin lambda, cyfluthrin beta, cypermethrin (I,II,III), fenvelerate and delta methrin); 60/100 mesh; acetonitrile (ACN), dichloromethane (DCM) and hexane were obtained commercially. Before use, florisil was activated at 550°C for 12 h and stored at room temperature. The required amount was pre-heated again at 130°C for 5 h prior to use in experiment. All other reagents used for analysis were analytical grade and supplied from Merck, US.

Sample preparation, extraction and cleanup

Raw milk sample: A method of Stieve (1983) was followed for this purpose. Briefly, 2 ml of homogenized milk, 5 g of activated florisil was added and stir until the sample became a freely flowing powder. Next, the column was prepared by adding 3 g of florisil to the already placed light petroleum ether. Further, the sample mixture was gently loaded to the column and eluted with 35 ml of petroleum spirit and DCM prepared in 4:1 ratio. Then elute was collected in a 150 ml conical flask and evaporated to dryness in a rotary vacuum evaporator to remove the DCM. The sample was washed thrice with 10 ml of hexane and again dried in vacuum. The samples were stored in 2 ml GC vials till analysis.

Fodder samples: A method of Luke and Doose (1983) was adopted for this purpose. Chopped fodder samples (15–25 g) were soaked in 300 ml of acetone:water (65:35 v/v) for 2 h. Thereafter, the mixture was blended at high speed for 2 min; subsequently the extract was filtered and transferred to a 1L separating funnel. To this, 200 ml of hexane:dichloromethane (DCM) (1:1 v/v) was added and shaken vigorously to separate the two layers. Next, lower aqueous phase was transferred to another separating funnel and the left upper organic phase was dried by passing through the bed of sodium sulphate. The separating funnel having aqueous phase was mixed with 10 ml of saturated sodium chloride solution and shaken vigorously for 30 s thereafter a similar extraction procedure was repeated as followed for aqueous phase. The extract was then concentrated using vacuum rotary evaporator and washed thrice with hexane. The final volume was raised to 3 ml with acetone, out of which 1 ml was subjected to the cleanup for organochlorines and pyrethroids and the remaining 2 ml used for the organophosphates (Luke 1983).

Cleanup for organochlorines and pyrethroids: To a sintered chromatographic column (22 mm), 50 ml of hexane was added and the column was prepared with 4 g of activated florisil followed by 2 g of sodium sulphate and again column was soaked with 15 ml hexane. Out of 3 ml extract obtained in acetone, 1 ml was diluted with 10 ml (10%) of acetone in hexane and transfer it to the florisil column. Column was eluted at the rate of 5 ml/min with 50 ml eluent (50% DCM: 1.5% ACN: 48.5% hexane v/v/v). The obtained elute was then concentrated to 1 ml. The extract was then analyzed for the organochlorine and synthetic pyrethroids by GC/ECD.

Cleanup for organophosphates: The opening of column was plugged with cotton and 2.5 g of celite-545 was added to it. Consecutively, 6 g mixture of celite-545 and activated carbon (4:1w/w) and 6g anhydrous Na_2SO_4 was added. Next, the column was soaked with 25 ml of DCM. Finally, the sample was placed on the column and eluted with 100 ml of DCM: Acetone (1:2, v/v) and thereafter concentrated using vacuum rotary evaporator. The final volume was raised to 1ml in hexane:acetone (9:1 v/v).

Ground water samples: A standardized protocol of AOAC (990.06) was followed to analyse all pesticides. Briefly, a 750 ml of water sample was taken in a 1 L of separating funnel and 150 g of NaCl and 75 ml of DCM was added to it. The admixture was shaken vigorously for 1 min and allowed to stand for 5 min basically to separate the organic and aqueous phases. Lower organic phase was passed through anhydrous Na_2SO_4 bed (20 g) and collected in a conical flask. This extraction procedure was repeated twice using 40 ml of DCM. The elute was then concentrated in rotary vacuum evaporator and the finally make up to 1 ml using hexane. The samples were then analysed in GC and quantified as mg/kg.

$$\text{mg/kg}= \frac{\text{Peak Area (sample)}}{\text{PeakArea (Standard)}} \times \frac{\text{Final Volume}}{\text{Weight of Sample}}$$

$$\times \text{ Concentration of standard} \times \frac{\text{Volume of Standard injected}}{\text{Volume of Sample injected}}$$

Gas chromatography (GC) instrumentation and conditions: A fully automatic GC/ECD/NPD system (GC-Shimadzu 2010) equipped with DB-1 (for organochorine estimation, I.D 30 m ×0.25 mm × 0.25 µm) and DB-5 (for organophosphate estimation, I.D 30 m × 0.25 mm × 0.25 µm) columns were used for the analysis of pesticides. A 1µl of sample was injected through the column using Nitrogen/Air as carrier gas (flow rate 12 ml/min) in 1:20 split ratio. The temperature of injector and detector were maintained at 280°C and 300°C respectively. The column oven temperature was maintained at 160°C and further raised from 160°C to 290°C @ 7°C/ min.

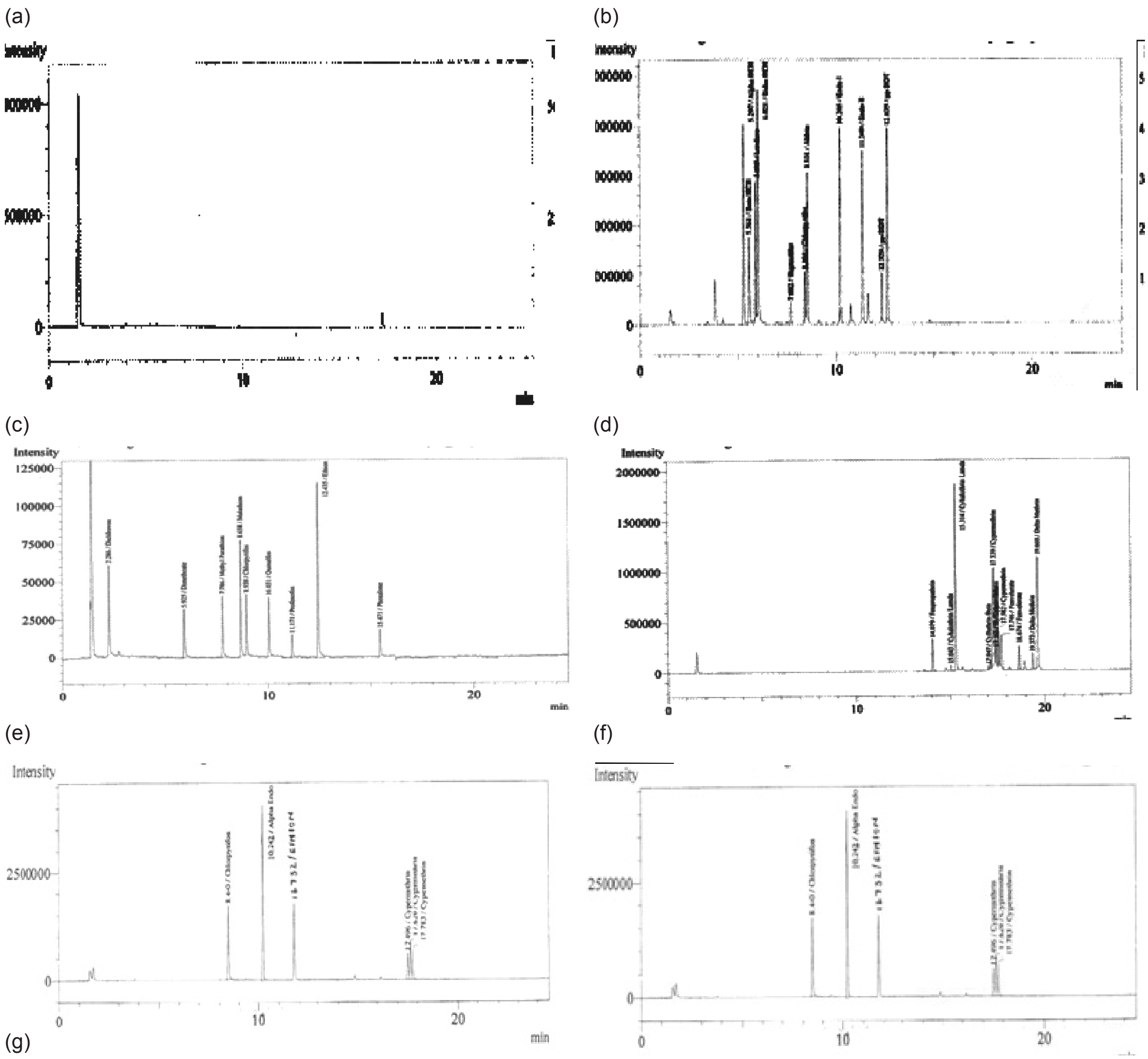
Method validation: Milk, water and fodder samples were validated by doing (three trials) recovery studies at fortification level of 0.01 mg/kg (Fig.1a-g). The obtained results are presented as in Table 1.

RESULTS AND DISCUSSION

The survey was conducted to collect the information regarding pesticide usage on vegetables (surplus and spoiled), and other crops utilized for animal feed/ fodder. In all, we have inquired 34 farmers from 8 different blocks of Jaipur district. The survey data revealed that 55% of farmers were using cypermethrin (in 0.5–1.0%

Table 1. Results of validation of method

Pesticides	Percent recovery ±SD (%), *n* = 3		
	Milk	Fodder	Water
Chlorpyriphos	94.89±1.89	95.61±4.89	83.31±2.98
Endosulfan-I	95.00±2.53	83.24±3.59	87.08± 1.40
Ethion	91.30±1.78	87.41±1.54	89.68±1.73
Cypermethrin	79.03±3.67	78.32±1.84	82.69±2.17



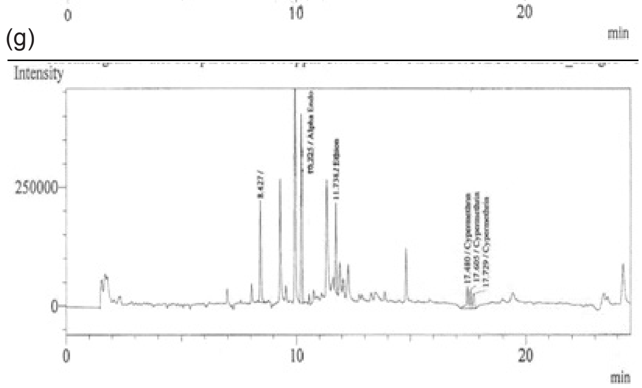


Fig. 1. Chromatograms showing (a) blank sample, (b) standards containing mixture of organochlorines, (c) organophosphates, (d) synthetic pyrethroids, (e) mixture containing OC, SP, OP, (f) milk and (g) water samples.

concentration), 35% melathion (1.0%), 30% chlorpyrifos (1.0%), 7.5% endosulfan (0.5–2.0%) and 2.5% phorates (no information was provided for its percent use) for vegetables and crops utilized for animal feeding. Further, we interpreted that among vegetable crops; tomato, cauliflower, and pea were the major vegetables identified on which the pesticides were maximally applied followed by groundnut, fruit (watermelon), gram and mustard. Similar supporting facts were also evident from the study of Gupta *et al*. 1998, where the authors found the presence of similar pesticides i.e endosulfan and chlorpyriphos in bottlegourd, cauliflower, cabbage and fenugreek samples. In addition to these, they also detected the presence of other pesticides including monocrotophos, fenvalerate, phosphamidon, HCH, DDVP, dimethoate, and methyl demeton.

Next, we found that at the detection limit of 0.01 mg/kg, the milk, fodder, and water samples were free from the pesticide residues (Table 2). This data in respect to milk samples was well supported from some previous findings where authors (Unnikrishnan *el al*. 1999, Surendra nath *et al*. 2000b, Gao *et al*. 2012) have not identified pesticide-DDT in bovine milk samples. Similarly, (Pandit *et al*. 2002,

Table 2. Pesticide residues (mg/ kg) in fodder samples including wheat straw, *bajra* stover and lucrene, raw milk and water samples collected from the farmers harbouring these animals in the different blocks of Jaipur district of Rajasthan from 2011–12

Source/ year of sampling	Pesticide analysed	Number and type of sample analysed	Detection level
Amer farmers	Organochlorines[a], Organophosphates[b],	(20) Fodder	BDL
(Kukas/Dhand/	Synthetic pyrethroids[c]	(20) Milk	BDL
Kunda/Khorameena)		(04) Water	
(2011–2012)			BDL
Shahpura farmers	Organochlorines, Organophosphates,	(20) Fodder	BDL
(Bilanderpur/Dewarala/Dhoblia)	Synthetic pyrethroids	(20) Milk	BDL
(2011–2012)		(04) Water	BDL
Kotputli farmers	Organochlorines, Organophosphates,	(20) Fodder	BDL
(Raghunathpur/Rawata)	Synthetic pyrethroids	(20) Milk	BDL
(2011–2012)		(04) Water	BDL
Bassi farmers	Organochlorines,Organophosphates,	(20) Fodder	BDL
(Bassi Industrial Area,	Synthetic pyrethroids	(20) Milk	BDL
Dindayal Nagar)		(04) Water	
(2011–2012)			BDL
Chomu farmers	Organochlorines, Organophosphates,	(20) Fodder	BDL
(Kaladera/Jaisinghpura/	Synthetic pyrethroids	(20) Milk	BDL
Biluchi)		(04) Water	
(2011–2012)			BDL
Chaksu farmers	Organochlorines, Organophosphates,	(20) Fodder	BDL
(Shivdaspura/Rampura)	Synthetic pyrethroids	(20) Milk	BDL
(2011–2012)		(04) Water	BDL
Phulera farmers	Organochlorines, Organophosphates,	(20) Fodder	BDL
(Hingonia/Karanshar/	Synthetic pyrethroids	(20) Milk	BDL
Dungari)		(04) Water	
(2011–2012)			BDL
Jamva Ramgarh farmers)	Organochlorines, Organophosphates,	(20) Fodder	BDL
(Mukundpura/Raithal/	Synthetic pyrethroids	(20) Milk	BDL
Jalsu/Disha)		(04) Water	
(2011–2012			BDL

BDL; below detectable level, Organochlorines[a], p,p'-DDT, endosulfan- (*alpha* and *beta),* HCH isomers (*alpha, beta, gamma* and *delta),* chlorpyrifos, heptachlor; Organophosphate[b], dichlrovos, dimethoate, methylparathion, malathion, chlorpyrifos, quinalfos, profenofos, ethion and phosalone; Synthetic pyrethroids[c], fenpropathrin, cyhalothrin lambda, cyfluthrin beta, cypermethrin (I,II,III), fenvelerate and delta methrin.

Balasubramuii *et al*. 1992, Pandit and Sahu 2007) affirmed that the levels of HCH and DDT in milk samples were substantially lower than they were reported earlier. The lower identification of these hazardous pesticides in the above described studies was due to increase in public awareness and regulatory authorities that have totally restricted their (DDT, HCH and endosulfan) usage. In contrast, Dhaliwal *et al.* (1977) and Ramakrishnan *et al.* (1982) had reported high levels of pesticides in milk and its products and that was basically due to indiscriminate use of these pesticides (DDT and HCH) during that period.

We presumed that the prevalent agro-climatic conditions (Semi arid eastern plain zone III A) of Jaipur region favor the fast degradation of pesticides and might be one reason for lower quantification of analysed pesticides in the fodder samples. According to the Solar energy centre, MNRE Indian Metrological Department, Jaipur region is showered with Direct, Global and Diffuse solar irradiance across the year and mean sunshine hours in Jaipur are from 6 to 19 hours. Also, in summers the temperature rises above 40°C making the climatic conditions adverse for the pesticide tenacity.

Metabolic physiology of animals also plays an important role that interferes in pesticides identification in milk, as lactating animals have the ability to remove these pesticide residues efficiently through urination and defecation. But the rate of bio-accumulation of these pesticides varies and depend upon their nature, actual load and quantity of produced milk daily (Bruce *et al*. 1965, Demott *et al*. 1966). Li *et al*. (1970) and Surendranath *et al*. (2000b) found that endosulphan present in the crops used for cattle feeding are not passed on to milk. To ascertain this fact, a study was conducted in which the authors have analysed the milk samples collected after 3 months of frequent endosulfan saprying (42.5 g/ha) on the fodder and found no pesticide residue on analysis samples (Ramesh and Vijyalaxmi 2002).

Earlier Arbeli *et al* (2007) reported that pesticides present in soil were metabolized through microbial activity and this might be one reason in present study as to why we have not identified pesticide residues in milk samples. Also, the volatilization and the rate of chemical and microbial degradation of these pesticides might be favored by high temperature under tropical regions as suggested by Simon *et al*. (1992) and Racke *et al*. (1997).

In addition, if traces of pesticides were still left un-degraded then there is much possibility that they were degraded by the microorganisms present in the ruminant gut especially the protozons. Kuches and Church (1971) reported that rumen protozoans have the ability to remove 95% of DDT from medium. Again, they have mentioned that these rumen microbes can degrade monocrotophos to 50 % of their original strength in just 12 h of incubation period.

Other miscellaneous factors that influence the degradation of pesticides include; soil, type of aquifer and depth of water table of the analysed region. Although, we have not estimated pesticides in these parameters but we hypothesized that these factors might have contributed to the degradation of pesticides in the environment. The depth of water table and type of aquifers present in the region might have decreased the chances of pesticides leaching to the groundwater and hence there are fewer possibilities for their indirect bioaccumulation in the foods especially those derived from animal sources.

Contamination in the milk with pesticide residues poses a significant health-risk to the public by drinking contaminated milk and its derived products hence it is important to timely analyse these pesticides in milk, fodder and water offered to milch animals in Jaipur region of Rajasthan. As contaminated feed and fodder are the main sources of pesticides in the animal body. In all analysed samples, the levels of pesticide residues (organochlorines, organophosphates and synthetic pyrethroids) were found below the detection limit. Therefore, we believe that use of such fodder is safe for animal consumption and their produced milk for human population.

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