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Body burden of pesticide residues vis-à-vis occurrence of reproductive disorders in dairy animals

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During last 60 years, a variety of pesticides have been used to increase India's agricultural productivity, the consumption of these has increased from 2,353 MT in 1955 to 43,630 MT in 2008 (GoI; www.indiastat.com). Owing to inadvertent use of pesticides, dairy animals in Punjab state have become highly vulnerable to their exposure, especially in the cotton belt districts known for high-pesticide usage (Ratnakaran *et al.* 2012). Organochlorine pesticides (OCP) are liphophic with low biological degradation rates due to which they have the tendency to accumulate in food chain (Bedi *et al.* 2005). Now OCPs have been replaced with organophosphorous pesticides (OPP) and synthetic pyrethroids (SP) in the developed countries, but in India OCPs are still being used to a certain extent (UNIDO 2009).

Till date, no study has been carried out to rule out whether or not widespread pesticide over-use is one of the etiological factors responsible for various reproductive disorders in dairy animals, but the research in developed countries has shown that environmental contamination of pesticides do produce reproductive disorders (Ghuman *et al*. 2013). The decline in fertility of dairy animals as well as humans in recent decades was related to environmental pollutants (Ghuman *et al*. 2012, Rolland *et al.* 2013). Hence, the present study was planned to assess the role of pesticides in various fertility problems of dairy animals.

Animals: The present study was carried out in 190 dairy animals (cattle and buffalo), of high-pesticide usage (HPU; District Muktsar) and low-pesticide usage (LPU; District Barnala, Ludhiana) areas of Punjab state. Higher cotton farming practices are present in HPU area and cotton crop is known as a pesticide-consuming crop (CACP 2006). From the LPU and HPU area, the animals were selected randomly and were either reproductively normal (estrus cyclic, pregnant) or abnormal (anestrus, repeat breeder, aborted, dystociac or prolapsed). Jugular vein blood (10 ml) samples collected from these animals and serum were stored at –20°C until analysis.

Pesticide standards: Analytical standards of organophosphorus pesticides, OPPs, OCPs and synthetic pyrethroids, SPs had 90–99% purity. The recovery of pesticides from fortified samples (50 and 100 ng/ml) was between 94 and 103%.

Extraction and clean-up technique for blood serum: Extraction was carried out as per Moreno *et al.* (2004) with modifications using 2 ml blood serum. The sample clean up was done as per USEPA method 3620B using florisil as adsorbent in glass column chromatography (USEPA 2003). Finally, sample was reconstituted in 2 ml mixture of n-hexane: acetone (1: 1 v/v).

Analysis: The clean-up extract (2 μl) was injected into DB–5 gas chromatography (GC) capillary column using auto injector. For OCP and SP analysis, an electron capture detector (ECD) was used and a flame thermionic detector (FTD) was used for OPPs. The parameters of GC for ECD and FTD were described earlier (Bedi *et al*. 2013). The confirmation of pesticide residues was done on GC-mass spectrometer equipped with a quadrupole mass analyser and operated by auto-sampler.

Statistical analysis: The concentrations of pesticide residues were summarized using arithmetic mean and standard deviation. A probability of $P<0.05$ was set as the significance level.

Analysis of blood or its products (serum, plasma) gives evidence of exposure of the animal body to pesticides and provides an indication of body burden of pesticide residues (Wessels *et al.* 2003). In the present study, almost similar proportion (15.1–17.5%) of dairy animals of LPU and HPU area were detected positive for pesticide residue(s) (Table 1). However, the serum pesticide residues were higher ($P<0.05$) in animals of HPU area (Table 1). This could be due to frequent pesticide sprays in HPU area as reported earlier (Ratnakaran *et al*. 2012). A study using serum samples have also detected higher prevalence of pesticide

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residues in dairy animals reared in pesticide-polluted area (Deepa *et al.* 2008).

The two most commonly detected pesticide residues in serum samples of dairy animals of present study were DDT and its metabolites (8.9%, 17/190), and chlorpyriphos (5.3%, 10/190) followed by butachlor (2.6%, 5/190), cyfluthrin (2.1%, 4/190), methyl parathion (1.6%, 3/190) and endosulphan sulphate (0.5%, 1/190; Table 1). The observations from survey of dairy farmers of HPU area have also suggested that chlorpyriphos is one of the most commonly used OPPs (Ratnakaran *et al.* 2012). Other OPPs (Monocrtophos, Profenophos, Ethion and Triazophos) used by the dairy farmers of LPU and HPU area were not detectable in the serum samples of dairy animals. This could be due to short biological half-life of OPPs (Ghuman *et al.* 2013). Intriguingly, the presence of DDT and its metabolites in maximum proportion of serum samples suggested the presence of DDT in the biological food-chain. It is pertinent to mention here that although the use of DDT is banned in India, but due to lack of suitable alternative for malaria control, it is still permitted to use up to 10,000 tonnes for vector control programmes (UNIDO 2009). The spraying of DDT for mosquito control in cattle shed was reported to contribute towards contamination of dairy milk (Battu *et al*. 1989). Moreover, the illegal diversion of DDT to agriculture could be one of the sources of detectable residues in agricultural commodities (Curtis and Lines 2000). In fact, in present study, the detection of pp-DDE, a metabolite of DDT, reflected past usage of DDT. In earlier studies in humans, pp-DDE, pp-DDT and pp-DDD residues were detected in breast milk samples (Bedi *et al*. 2013).

The proportion of dairy animal positive for pesticide residues and exhibiting normal estrus cyclicity or anestrus was similar in animals of LPU and HPU area (16.1–16.7%, P>0.05, Table 1). Moreover, serum pesticide residues were similar between estrus cyclic and anestrus animals (57.6±53.7 and 45.4±56.8 ng/ml, respectively, P>0.05, Table 1). However, the observed higher concentrations of pesticide residues in normal cyclic animals may ultimately become a causative factor behind the occurrence of repeat breeding conditions in these animals. In fact, compared to normal cyclic or anestrus animals, the proportion of animals positive for residues as well as suffering from repeat breeding syndrome tended to be higher (24%) along with higher serum concentrations of pesticide residues (70.1±82.8 ng/ml, P>0.05, Table 1). In contrast, the proportion of animals positive for a pesticide residue and exhibiting pregnancy was very less (4%), and the serum pesticide residues were also low (11.8±0.5 ng/ml) compared to estrus cyclic and anestrus animals (P>0.05, Table 1). These findings of pregnant animals in comparison to anestrus and repeat breeder animals suggested that serum

Table 1. Serum concentrations (mean±SD) of pesticide residues in dairy animals, with normal or abnormal reproductive status, reared in low-pesticide usage (LPU) and high-pesticide usage (HPU) areas

Observations		LPU area, n=53	HPU area, n=137
Animals positive for any pesticide residue		16.8%; 56.1±62.1 ng/ml	
		15.1%	17.5%
		[a]27.5±21.0 ng/ml	[b]65.6±68.5 ng/ml
Pesticides		Serum conc	Serum conc
OPP	Chlorpyrifos	10.6±2.2	22.3±26.3
	Methyl parathion	11.6	9.9±3.7
OCP	DDT and its metabolites	26.7±23.1	65.6±93.3
	Endosulphan sulphate	ND*	24.1
	Butachlor	ND	87.7±38.3
SP	Cyfluthrin	ND	64.6±20.6
Animals with normal reproductive status		41.5% (22/53)	43.1% (59/137)
Animals with normal status and positive for a residue		13.6% (3/22)	11.9% (7/59)
		37.5±35.0 ng/ml	61.4±58.4 ng/ml
Estrus cyclic		12.5%	17.5%
		44.5±46.5 ng/ml	61.4±58.4 ng/ml
Pregnant		16.7%	0%
		11.8±0.5 ng/ml	0.0±0.0 ng/ml
Animals with abnormal reproductive status		58.5% (31/53)	56.9% (78/137)
Animals with abnormal status and positive for a residue		16.2% (5/31)	21.8% (17/78)
		[a]21.5±6.1 ng/ml	[b]67.4±73.9 ng/ml
Anestrus		8.3%	22.2%
		21.1±0.0 ng/ml	51.4±63.9 ng/ml
Repeat breeder		14.3%	25.6%
		18.5±0.0 ng/ml	74.8±85.1 ng/ml
Other abnormal conditions (abortion, dystocia, genital tract infection or genital tract prolapse)		25%	14.3%
		17.0±9.2 ng/ml	29.1±19.7 ng/ml

[a vs b] P<0.05, within a row; *ND, non-detectable; OPP, organophosphorus pesticide; OCP, organochlorine pesticide; SP, synthetic pyrethroid.

concentrations of pesticide residues may play a role in occurrence of reproductive disorders viz. anestrus and repeat breeding syndrome.

The categorization of pesticide residue positive estrus cyclic, anestrus and repeat breeder animals based upon their rearing area revealed that major proportion (77.8, 80.0 and 90.9%, respectively) of these groups of animals were present in the HPU area and they had higher serum pesticide residue concentrations in comparison to their counterparts in LPU area (P>0.05, Table 1). The higher proportion of pesticide positive estrus cyclic animals of HPU area may hold their future as repeat breeders. Thus, these observations suggested that reproductive disorders in dairy animals are associated with serum pesticide residue concentrations and animals reared in HPU area are more prone to failure of normal reproductive conditions. In addition, serum concentrations of specific pesticide residues, viz. chlorpyriphos, methyl parathion, DDT and its metabolites, endosulphan sulphate, butachlor and cyfluthrin also tended to be higher in dairy animals of HPU area in comparison to their respective counterparts of the LPU area (Table 1).

Chronic exposure to environmental pollutants would be expected to lead to their accumulation in body fat and serum and consequently in follicular fluid (Wolff 1983). Organochlorine pesticides found in the bovine ovary as well as follicular fluid could be detrimental to granulosa cells and to the plasma membranes of oocytes resulting in impaired fertility (Kamarianos *et al.* 2003). The extent of damage to fertility by the observed level of pesticide residues in the present study can be extrapolated from the fact that a mixture of pesticides even at 1.0 ng/ml exerted adverse impact on bovine oocyte maturation and embryonic development (Pocar *et al.* 2001). The maturation stages of oocyte and early embryo have susceptibility even towards background doses of pesticide residues (Pocar *et al.* 2001). Moreover, quality and viability of cumulus cells decreased, developmental competence of exposed oocytes got affected and the blastocyst rate and quality reduced in a dose-dependent manner (Campagna *et al.* 2001).

The proportion of dairy animals exhibiting other reproductive disorders, viz. abortion, dystocia, genital tract infection or genital tract prolapse was 18.2% (Table 1). However, their serum pesticide residue concentrations tended to be lower (25.0±15.8 ng/ml) compared to those observed in estrus cyclic, anestrus or repeat breeder animals (Table 1). Probably, the occurrence of abortion, dystocia, genital tract infection or genital tract prolapsed is associated with other causative conditions in comparison to the role of serum concentrations of pesticide residues.

Similar proportion (16.8%) of dairy animals of low-pesticide consumption and high-pesticide consumption area were positive for pesticide residue(s), however, serum concentrations of pesticide residues were high in dairy animals of the high-pesticide consumption area (27.5±21.0 vs 65.6±68.5 ng/ml). High proportion of repeat breeder dairy animals positive for pesticide residues (24%; 70.1±82.8 ng/ml) compared to low proportion of pregnant animals positive for pesticide residues (4%; 11.8±0.5 ng/ml) suggested the role of body burden of pesticide residues as a causative factor underlying their abnormal reproductive status.

SUMMARY

For assessing the association between body burden of pesticide residues and occurrence of reproductive disorders in dairy cattle and buffalo, random blood samples were collected from reproductively normal or abnormal bovines (190) reared in high pesticide usage (HPU) and low pesticide usage (LPU) area of Punjab state. Multiple pesticide residue analysis was done using gas chromatography (GC) and GC-mass spectrometry (*GC-MS*). Overall, about 16.8% bovines were detected positive for pesticide residues (56.1±62.1 ng/ml). The two most commonly detected pesticide residues in blood serum samples of bovines were DDT and its metabolites and chlorpyriphos. Serum pesticide residues were not different between the estrus cyclic and anestrus bovines. Compared to normal estrus cyclic or anestrus bovines, the proportion of bovines positive for pesticide residues as well as suffering from repeat breeding syndrome tended to be higher (24%) along with higher serum pesticide residues (70.1±82.8 ng/ml). In contrast, the proportion of bovines positive for a pesticide residue and exhibiting pregnancy was very less (4%), and their serum pesticide residues was also low (11.8±0.5 ng/ml). In brief, these findings suggested that serum concentrations of pesticide residues may play a role in occurrence of reproductive disorders.

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