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In utero exposure of neonatal buffalo calves to pesticide residues and the alterations within their reproductive tract

Karanpreet Kaur[a], Sarvpreet Singh Ghuman[a], Opinder Singh[b], Jasbir Singh Bedi[c,*], Jatinder Paul Singh Gill[c]

[a] Department of Veterinary Gynaecology and Obstetrics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141004, India
[b] Department of Veterinary Anatomy, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141004, India
[c] School of Public Health and Zoonoses, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141004, India

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ABSTRACT

In utero exposure of neonates to pesticide residues could be damaging to the reproductive tract. Hence, the present study assessed the circulating concentrations of pesticide residues in buffalo and their neonatal calves as well as in the reproductive tract tissue samples of same calves. Also, histopathological alterations were revealed in the reproductive tract of calves. Pesticide residues were high ($P < 0.05$) in the reproductive tract of calves (119.5 ± 20.2 ng/g, 35% positive) in comparison to their blood (32.1 ± 8.4 ng/ml, 15% positive) or blood of their dams (41.5 ± 8.3 ng/ml, 25% positive). The number of histopathological alterations were high ($P < 0.05$) in the reproductive tract of a calf contaminated with high concentrations of pesticide residues (3.43 ± 1.29) in comparison to a tract positive for low residue concentrations (1.57 ± 0.60) or pesticide negative tract (0.28 ± 0.10). In conclusion, *in utero* exposure of neonatal buffalo calves to pesticide residues may be associated with damaging alterations in their reproductive tract.

1. Introduction

Although, pesticide consumption has declined in India due to Integrated Pest Management Scheme, but still there is a widespread contamination of environment due to persistant nature of some classes of pesticides and their inadvertent usage (Directorate of Plant Protection Quarantine and Storage, 2014). At the same time, the decline in fertility in humans and animals over the recent years is being attributed to environmental pollutants including pesticides (Harley et al., 2008; Ghuman et al., 2013). *In utero* exposure of pesticide residues can damage the reproductive axis of foetus by mimicking or antagonizing the action of endocrine hormones (Kandarakis et al., 2009). Furthermore, during late pregnancy, the body reserve of pesticide residues accumulated during the life-time exposure of dam get mobilized due to negative energy balance induced lipolytic activity, thus exposing the developing foetus to higher concentrations of residues (Magnarelli, 2012). During the critical period of foetal development, even a transient exposure of pesticide residues, at concentrations much lower than that required for producing toxic impact in adults, can cause irreversible damage to rapidly dividing primordial germ cells in the gonads and this is manifested in form of infertility in adult life (Uzumcu and Zachow, 2007). Considering the potential adverse impact of pesticide residues *in utero* and hence to save the future of dairy industry, the present study in buffalo was aimed to find out the circumstantial evidence regarding the association of circulating pesticide residue concentrations in the dam and neonatal calf, with the histopathological alterations in the reproductive tract of neonatal calf.

2. Materials and methods

2.1. Sampling

The present study was carried out in 100 normal calving buffalo and their calves. For pesticide residue analysis, buffalo and their calves, at the time of calving, were sampled for 10 ml jugular vein blood. Also, the reproductive tract of calves were removed and a small portion of the tract was kept in 10% Neutral Buffered Formalin for histopathology and the remaining was stored at −20 °C for pesticide residue analysis. All the procedures were performed in compliance with laws and guidelines of 'Institutional Animal Ethics Committee (License no. VMC/14/160-185, dated 17.01.2014)'.

* Corresponding author.
E-mail address: bedijasbir78@gmail.com (J.S. Bedi).

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2.2. Pesticide standards

Analytical standards of Organochlorine pesticides, OCPs (HCH and its metabolites, Methoxychlor, Heptachlor, α-Chlordane, Mirex, Aldrin, Fipronil, Butachlor, Dieldrin, DDT and its metabolites, Endrin, Endosulfan and Toxaphene), Organophosphorus pesticides, OPPs (Chlorpyrifos, Monocrotophos, Dimethoate, Phorate, Fenitrothion, Parathion-methyl, Malathion, Fenamiphos, Profenphos, Ethion, Triazophos, Phosalone and Quinolphos) and Synthetic pyretheroids, SPs (Cypermethrin, Permethrin, Cyfluthrin, Cyhalothrin, Deltamethrin and Fenvalerate) were used to detect the residues of these pesticides in the samples.

2.3. Method validation

The 'Limit of Detection' and 'Limit of Quantification' of extraction method were 1 ng/g and 5.4 ng/g, respectively, for OCPs and OPPs, and 2.3 ng/g and 13.0 ng/g for SPs, respectively. The mean recoveries obtained by spiking samples with multiple pesticides in concentrations ranging from 50 to 100 ng/g were 70–80%.

2.4. Extraction and clean up technique for the detection of pesticide residues

As per the method of Sharma (2007), 5 g of each reproductive tract sample was finely ground thrice with a homogenizer in 60:40 hexane:acetone. After each homogenization, filtration was done to obtain clear hexane extracts, which were further concentrated for fat determination. The fat aliquot was extracted using acetonitrile saturated in hexane followed by 15% dichloromethane in hexane, subjected to florisil clean up and used for estimation of OCPs, SPs and moderately polar OPPs. Aqueous layer left following dichloromethane-hexane partitioning was further extracted thrice with dichloromethane. Extract was concentrated to near dryness, final volume made up to 5 ml with 10% acetone in hexane and directly used for estimation of relatively polar OPPs. For florisil clean up, the sample was eluted using three different eluants at 2 ml/min [Eluant 1: 20% dichloromethane and 80% hexane (v/v); Eluant 2: 50% dichloromethane, 0.35% acetonitrile and 49.65% hexane (v/v) and Eluant 3: 50% dichloromethane, 1.5% acetonitrile and 48.5% hexane (v/v)]. Eluate was concentrated using vacuum evaporator and residues were reconstituted in hexane:acetone (1:1) mixture and analyzed.

As per the method of Gill et al. (1996), 1 ml blood samples of each buffalo and calf were extracted thrice with hexane:dichloromethane (9:1) after addition of glacial acetic acid. Organic extracts were evaporated and concentrated to 1–2 ml using vacuum evaporator, subjected to florisil clean up and eluted with hexane, 1:9 hexane:dichloromethane, 6% diethylether in hexane, 15% diethylether in hexane, 50% diethyl ether in hexane and finally diethylether. Eluate was collected and evaporated to dryness. Finally, sample was reconstituted in n-hexane:acetone (1:1) and analyzed.

2.5. Analysis

Clean up extract (1–2 μl) was injected into Gas Chromatograph (GC) capillary column equipped with 'Electron Capture Detector' for OCP and SP detection or 'Flame Thermionic Detector' for OPP detection. Analyte was identified by comparing the retention times and peak height/area with the reference standards run under similar operating conditions (Bedi et al., 2013). The confirmation of pesticide residues was done by GC–Mass Spectrometer (MS), in which a characteristic mass spectrum was obtained based on mass-charge ratio of a compound. The reagent and sample blank were extracted and analyzed in triplicate to negate the false peaks in common.

2.6. Histopathology procedures

The tissue samples from reproductive tract (stored in 10% NBF) of buffalo calves were cut into sections of 4–5 μm thickness, processed for paraffin sectioning by Acetone–Benzene schedule and stained using Hematoxylin & Eosin for morphological studies of tissues and using Masson's Trichrome for collagen fibres (Luna, 1968).

2.7. Statistical analysis

Pesticide residue concentration data was summarized as Mean ± SE. Within or between group differences in samples positive for pesticide residues, pesticide residue concentrations and histopathological alterations were obtained by One-Way Analysis

Table 1
Pesticide (OCP: organochlorine pesticides, OPP: organophosphorus pesticides, SP: synthetic pyrethroids) residue concentrations (Mean ± SE) in the blood samples (n = 100) of buffalo and neonatal calf as well as in the calf reproductive tract.

	Buffalo blood (ng/ml)	Calf blood (ng/ml)	Calf reproductive tract (ng/g)
Cumulative results			
Pesticide residue conc	41.5 ± 8.3a,c	32.1 ± 8.4b	119.5 ± 20.2d
Samples +ve for a residue	25%#	15%#	35%#
Samples +ve for >1 residue	24%	26.6%	37.1%
Concentrations of a pesticide residue category			
OCPs (n = 38*)	48.5 ± 8.7a (11)	38.0 ± 5.9a,p (9)	117.9 ± 28.1b (18)
OPPs (n = 25*)	38.0 ± 14.6a (7)	30.9 ± 12.8a,r (6)	85.7 ± 11.7b,r (12)
SPs (n = 12*)	30.5 ± 17.9 (3)	65.3 ± 46.3q,s (2)	236.2 ± 90.8s (7)
Concentrations of a specific pesticide residue			
OCPs			
DDT metabolites	32.4 ± 11.5 (8)	33.0 ± 19.4 (8)	99.9 ± 27.7 (13)
γ-HCH	41.6 (1)	15.3 (1)	458.5 ± 79.6 (3)
β-HCH	ND	ND	40.7 (1)
Endosulfan	20.8 ± 12.2 (3)	18.8 ± 11.0 (3)	88.5 ± 52.0 (3)
OPPs			
Ethion	21.3 (1)	22.9 (1)	70.1 ± 35.0 (4)
Chlorpyriphos	24.2 (1)	ND	90.2 ± 42.4 (2)
Methylparathion	44.7 (1)	16.2 (1)	58.1 (1)
Malathion	48.4 ± 24.2 (4)	33.7 ± 16.8 (4)	73.6 ± 33.4 (5)
SPs			
Cypermethrin	44.8 ± 26.3 (3)	65.5 ± 46.7 (2)	192.2 ± 80.8 (6)
Permethrin	ND	ND	501.2 (1)

[a vs b] $P < 0.05$, within a row; [p vs q, r vs s, *] $P < 0.05$, within a column; [#, c vs d] $P < 0.05$, within a row; Figures in parenthesis indicate samples positive; ND, non-detectable.