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Some Reproductive and Health Aspects of Female Buffaloes in Relation to Blood Lead Concentration

¹W.M. Ahmed, ¹A.R. Abd El-Hameed and ²F.M. El-Moghazy

¹Department of Animal Reproduction and A I,

²Department of Parasitology and Animal Diseases, Veterinary Research Division,
National Research Centre, P.O. Box 12622, El-Tahrir Street, Dokki, Giza, Egypt

Abstract: This study was designed to associate Blood Lead Concentration (BLC), reproductive disorders and oxidant/antioxidant status in female buffaloes reared besides high ways. Animals were clinically examined and blood samples were collected from 30 non pregnant female buffaloes for assaying of lead and some oxidant/antioxidant values. According to BLC, animals were divided into two groups. The high BLC group showed high incidence of reproductive disorders in form of inactive ovaries, delayed puberty, endometritis, repeat breeding, mastitis, persistent corpora lutea and abortion. Malondialdehyde (MDA) and Nitric Oxide (NO) values increased, while, Total Antioxidant Activity (TAA), Superoxide Dismutase (SOD), Glutathione Reduced (GR) and Selenium (Se) values decreased in buffaloes of high BLC. It was concluded that there is a tight relationship between blood lead concentration, reproductive disorders and oxidant/antioxidant imbalance in buffaloes.

Key words: Buffalo, reproductive disorders, lead, oxidant/antioxidant status, parasites

INTRODUCTION

Buffalo is the main dairy animal of Egypt, despite this species suffers from several reproductive disorders, especially delayed puberty, silent heat, ovarian inactivity, endometritis and repeat breeding (Ahmed *et al.*, 2006).

Lead is a pervasive and widely distributed environmental pollutant with no reported beneficial effects in man and animals. Lead poisoning is more common in farm animals and ruminants are considered the most susceptible animals to its toxic effects (Radostits *et al.*, 2000; Abd El Hameed, 2003).

High blood lead levels in animals have been reported from various parts of the world including India (Swarup *et al.*, 2005) and Egypt (Khalaf Allah and Abd El-Aal, 1999), particularly in urban localities.

Lead is a well-known reproductive toxin in male (Milnes *et al.*, 2006) and female animals. In female rats, lead exposure was associated with delayed sexual maturity, irregular estrus and reduced numbers of corpora lutea (Iavicoli *et al.*, 2006) and increased risk of spontaneous abortion (Bellinger, 2005). In farm animals, lead exposure induced, abortion, poor pregnancy rate and increased service interval in female goats (Abd El-Hameed, 2003), endometritis in ewes (Stoev *et al.*, 1997) impaired fertility in cows (Buhatel *et al.*, 1985; McEvoy and McCoy, 1993) and poor conception rate, reduced detection of heat and increased service interval in buffalo- cows (El Tohamy *et al.*, 1997; Ahmed, 2006).

Corresponding Author: Dr. Wahid M. Ahmed, Department of Animal Reproduction and A I,
Veterinary Research Division, National Research Centre, P.O. Box 12622, El-Tahrir Street,
Dokki, Giza, Egypt

It was reported that lead exposure causes generation of Reactive Oxygen Species (ROS) and increased the level of lipid peroxidation (Upasani *et al.*, 2001). This condition leads to disrupting of the delicate pro-oxidant/antioxidant balance within cell, alteration of antioxidant defense system in animals and aggravates its pathogenesis (Hsu and Guo, 2002).

Parasitic infection particularly in tropical and subtropical countries represents an important cause of direct and indirect losses in farm animals. The effect of parasitism is not easily evaluated as the infection runs usually in a sub-clinical longstanding course. However, the most important outcomes due to parasitism are retardation of growth, loss of body weight, increased susceptibility to other diseases and increased mortality rate leading to considerable financial losses. Interference with reproductive function is also recorded in affected animals in the form of neonatal mortality, delayed puberty, abortion and low reproductive performance in mature animals (Barakat *et al.*, 2001).

This study was designed to find the possible association between the concentration of lead in blood of buffaloes reared near highways and the occurrence of reproductive disorders and parasitic infection. In addition, investigating oxidant/antioxidant status of these animals was another target.

MATERIALS AND METHODS

Animals

Field visits were carried out to small holder buffalo farms nearby the high ways at Lower Egypt during the period from September 2004 to June 2006. A total number of 30 non pregnant female buffaloes were selected during the breeding season (September-March) to carry out the current study.

Experimental Design

Clinical Examination

Owners complain and case histories of the experimental buffaloes were recorded. Animals were clinically examined and Body Condition Score (BCS) was recorded on scale of 1 (very thin) to 5 (very thick) as recorded by Ahmed *et al.* (1999). Rectal palpation was carried out and the reproductive status of animals was confirmed later on by progesterone analysis (ELISA, data are not shown in this study).

Sampling

Two types of blood samples were taken from the Jugular vein. The first sample was taken in nitric acid washed heparinized tubes for analysis of BLC, GR and Se. The second sample was taken in plane tubes for separation of serum (3000 Xg/15 min at 4°C) for analysis of MDA, NO, TAA and SOD. In addition, fecal samples were collected for parasitological examination.

Laboratory Analysis

Blood Lead Analysis

Blood lead analysis in the sample was performed by deproteinization of 0.1 mL blood using 1.1 mL precipitating reagent consists of 949 mL of deionized water, 50 mL nitric acid and 1 mL Triton X-100. Samples were left for 15 min and centrifuged for 5 min at 3000 rpm. the obtained supernatant was used for measuring lead by graphite furnace atomic absorption spectrophotometry at a wave length of 283.7 nm (Yee *et al.*, 1994).

Oxidant/Antioxidant Markers

MDA (Sato, 1987), NO (Montgomery and Dymock, 1961), TAA (Koracevic and Koracevic, 2001), SOD (Nishikimi *et al.*, 1972) and GR (Beutler, 1963) values were determined calorimetrically by enzymatic reactions using chemical kits from Diagnostic, Egypt. Se in whole blood was determined by graphite furnace atomic absorption spectrophotometry at a wave length of 196 nm.

Fecal Examination

Fecal examination was carried out as outlined by Soulsby (1969)

Statistical Analysis

Data were computed and statistically analyzed used Student's t-test (Snedecor and Cochran, 1980). According to blood lead concentration animals were divided into 2 groups. Those with concentration < 20 or > 20 $\mu\text{g dL}^{-1}$ as previously suggested by Swarup *et al.* (2005).

RESULTS

Table 1 shows data pertaining to BLC in the examined female buffaloes, which were reared at small holder farms nearby high ways at Lower Egypt. The mean BLC ($\mu\text{g dL}^{-1}$) was 15.84 ± 0.49 in low lead group and 24.55 ± 0.75 in the high lead group.

Female buffaloes with high BLC have obviously poor body condition and revealed significantly inferior BCS ($p < 0.01$) as compared with low BLC group (2.0 ± 0.22 versus 3.10 ± 0.22 on scale of 1-5).

During the breeding season, obviously high incidence of animals in the high BLC group having bilateral smooth inactive ovaries (45.00%), delayed puberty (20.00%), endometritis, repeat breeding and mastitis (10.00% for each), persistent corpus luteum and abortion (5.00% for each) as compared with the low BLC group. The highest BLC was detected in animals suffered from repeat breeding and mastitis ($31.00 - 35.00 \mu\text{g dL}^{-1}$).

Regarding the oxidant/ antioxidant status, data presented in Table 2 revealed significant increases ($p < 0.01$) in MDA and NO in buffaloes of the high BLC as compared to the low BLC group. On the other hand, antioxidant markers, especially TAA, SOD, GR and Se tend to decrease in buffaloes had high BLC.

Table 1: Effect of blood lead concentration on the health and reproductive status of female buffaloes reared nearby high ways at Lower Egypt

Parameters	Low BLC group (NO = 10) [#]	High BLC group (NO = 20)
Blood lead concentration ($\mu\text{g dL}^{-1}$)		
Mean	15.84	24.55
SE	0.49	0.75
Range	0.00-20	20.00-35
BCS	3.10 ± 0.22	$2.0 \pm 0.22^{**}$
Reproductive status (%)[†]		
Normal cyclic animals	90.00	5.00
Bilateral smooth inactive ovaries	10.00	45.00
Delayed puberty	00.00	20.00
Endometritis	00.00	10.00
Persistent corpus luteum	00.00	5.00
Repeat breeding	00.00	10.00
Mastitis	00.00	10.00
Abortion	00.00	5.00

[#] NO.: Number of animal/group SE: Standard Error ^{**}: $p < 0.01$; BLC: Blood Lead Concentration, BCS: Body Condition Score (on 1-5 scale), [†]: Two animals revealed more than one disorders

Table 2: Oxidant /antioxidant status of female buffaloes reared nearby high ways at Lower Egypt in relation to BLC (Mean \pm SE)

Oxidant/antioxidant markers	Low BLC group (NO = 10) [#]	High BLC group (NO = 20)
Malonaldehyde (mmol mL^{-1})	1.89 ± 0.06	$5.18 \pm 0.79^{***}$
Nitric oxide ($\mu\text{mol L}^{-1}$)	16.51 ± 0.94	$29.54 \pm 2.13^{***}$
Total antioxidant activity (mmol L^{-1})	1.39 ± 0.08	$0.64 \pm 0.04^{***}$
Superoxide dismutase (μmL^{-1})	336.40 ± 6.81	$298.29 \pm 2.79^{***}$
Glutathione reduced (mmol L^{-1})	6.98 ± 0.23	$1.59 \pm 0.19^{***}$
Selenium ($\mu\text{g L}^{-1}$)	135.36 ± 0.39	$118.90 \pm 2.04^{***}$

[#] NO: Number of animal/group SE: Standard Error ^{***}: $p < 0.001$; BLC: Blood Lead Concentration

Table 3: Parasitic infestation in female buffaloes reared nearby high ways at Lower Egypt in relation to BLC (%)

Parasitic infestation	Low BLC group (NO = 10) [#]	High BLC group (NO = 20)
Coccidia	10.00	20.00
Trichostrongylus	00.00	15.00
Fasciola	10.00	00.00
Babesia	00.00	10.00
Mange	10.00	20.00

[#]NO: Number of animal/group BLC: Blood Lead Concentration

From the parasitological point of view, Table 3 shows high incidence of parasitic infestation in the high BLC group as compared with the low BLC group, especially with coccidia, trichostrongylus, babesia and mange.

DISCUSSION

Lead has been recognized as a major environmental pollutant with diverse deleterious effects in man and animals.

In the present study, buffalo cows reared besides high ways at Lower Egypt showed a significant increase in BLC. This finding coincide with those reported by Swarup *et al.* (2005) in cows reared at areas around different industrial activities as well as by Khalaf-Allah and Abd El-Aal (1999) in sheep grazing in industrized area polluted with lead and by Ward and Savage (1994) in horses exposed to traffic emission. The higher lead levels in animals reared around such industrial activities are mainly due to ingestion of pasture contaminated with lead as well as inhalation of lead particles (Okada *et al.*, 1997; Abd El-Hameed, 2003). However, not all exposed animals showed high BLC whereas, there are many factors affecting lead toxicity such as individual variations, age, nutritional status and concentration of calcium, iron and Vitamin D in the blood (Abd El-Hameed, 2003).

In the current study, it was found that female buffaloes with high BLC showed poor BCS, high incidence of reproductive disorders, imbalance oxidant/antioxidant status and parasitic infestation.

The poor BCS in high BLC animals could be attributed to the appetite-depressant effect of lead (Hammond and Succop, 1995) and in tern decreased feed consumption and conversion rates. Moreover, the condition get confirmed by Huseman *et al.* (1992) who added that lead causes an inhibition of the release of pituitary growth hormone.

In the present study, female buffaloes have high BLC showed higher incidence of inactive ovaries, delayed puberty, endometritis, persistent corpora lutea, repeat breeding, mastitis and abortion as compared with the low BLC group. Similarly, a variety of adverse reproductive outcomes such as spontaneous abortion, impaired fecundity and sterility was reported in exposed animals (Foster *et al.*, 1996 ; Pace *et al.*, 2005).

Regarding the high incidence of ovarian inactivity in buffalo-cows with high BLC herein, it was reported that lead induced reproductive toxicity and affect ovarian function and fertility of exposed animals, mainly due to both central and gonadal functional disturbances (Ronis *et al.*, 1996; Sant'Ana *et al.*, 2001). Centrally, due to reduction of hypothalamic GnRH levels (Camorato *et al.*, 1993), decreased LH and FSH concentrations (Batra *et al.*, 2004) and interference with pituitary hormone release via interaction with calcium-dependant secondary messengers system, which mediates hormone release from secretory granules storage (Klein *et al.*, 1994). At gonadal level, lead has a direct effect, through affecting germinal epithelium (Stoev *et al.*, 1997), decreased gonadal weight or even act synergistically to reduce DNA gonadal content (Corpas and Antonia, 1998) and disturbed folliculogenesis due to its tissue accumulation (Taupeauet *et al.*, 2001).

The occurrence of delayed puberty in high BLC buffaloes in this study agree with the findings recorded in lead exposed goats (Abd El-Hameed, 2003), rats (Dearth *et al.*, 2002) and mice (Iavicoli *et al.*, 2006). The condition was attributed to the toxic effect on hypothalamic-pituitary-

gonadal axis and decreased levels of hormones involved in the growth and reproduction. This effect was reported when animals were exposed either during pre- or post- natal periods (McGivern *et al.*, 1991). Moreover, it was found that this delay is associated with suppressed serum levels of Insulin-like growth factor-1, LH and estradiol 17 β (Dearth *et al.*, 2002; Pine *et al.*, 2006).

In this study, the highest BLC was found in animals suffering from repeat breeding and mastitis. In this respect, poor pregnancy rate and increase of service period were the more pronounced reproductive disorders in lead exposed female goats (Abd El-Hameed, 2003) and buffaloes (El-Tohamy *et al.*, 1997). These defects were attributed to the effect of lead on the hormonal function and the genital tract of exposed animals even in spite of occurrence of normal estrus (Gorbel *et al.*, 2002). In the same time, adverse affects in all items of reproduction including conception, implantation of fertilized ova, fetal survival and growth in rats exposed to lead were recorded (Abdalla *et al.*, 1992; Robert *et al.*, 2004).

Abortion in buffaloes with high BLC in this study coincides with the findings of Frape and Pringle (1984) in cows and Abd El-Hameed (2003) in goats. The condition was attributed either to the decline of progesterone (Abd El-Hameed, 2003) or as a result of crossing of lead through placenta (Neathery and Miller, 1975), reaching to fetus itself or it induced placentitis and fetal death (O Hara *et al.*, 1995).

In the present work, buffaloes cows with high BLC showed imbalance oxidant/ antioxidant status as indicated by increase value of MDA and NO and decreased SOD, GR, TAA and Se as compared to the low BLC group. Similar results were found by Orhan *et al.* (2004) in battery plant workers and Hande *et al.* (1998) in rats. Tabacova *et al.* (1994) added that exposure to lead enhance the development of pregnancy complications by increasing lipid peroxidation via depletion of reduced glutathione reserves. The γ -Aminolevulinic Acid Dehydrase (ALAD) is highly sensitive to the toxic affects of lead (Farant and Wigfield, 1982). The accumulation of ALA induced generation of ROS (Hermes-Lima, 1995). Also, lead has a high affinity for sulfhydryl (SH) group (Valle and Ulmer, 1972) and it can alter antioxidant activities by inhibiting functional SH group in ALAD, SOD, CAT and Glutathione Peroxidase (GPX) enzymes (Chiba *et al.*, 1996). Moreover, decreased Se associated lead may increase the susceptibility of the cell to oxidative stress and decreased SOD, GR, GPx activity (Schrauzer, 1987; Othman and El-Missiry, 1998).

It has been recorded that lead exposure causes immunosuppression as it affects both cellular (Brar *et al.*, 1995) and humeral (Hoffman *et al.*, 1995) immunity. The condition is intensified by lead-induced oxidative stress (Ercal *et al.*, 2000). This immune suppression resulted in increase parasitic infestation by the different recorded parasites (coccidia, trichostrongylus, babesia and mange) in buffaloes of the high lead group in this study.

From this study, it could be concluded that there is a tight relationship between blood lead concentration, reproductive disorders and oxidant/antioxidant imbalance in buffaloes. It is recommended to build animal farms far away from industrialized areas and high ways.

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