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

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

Department of Animal Reproduction and A.I., Department 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 (lavrcic 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 (Buhateil 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 (Burakat 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 9:49 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 spectrophotometery at a wave length of 283.7 nm (Yee et al., 1994).

Oxidant/Antioxidant Markers
MDA (Satoh, 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 spectrophotometery 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 μg dL⁻¹ as previously suggested by Swamp 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 (μg 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 (<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 μg 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, CR 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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Low BLC group (NO = 10)*</th>
<th>High BLC group (NO = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood lead concentration (μg dL⁻¹)</strong></td>
<td>15.84 ± 0.49</td>
<td>24.55 ± 0.75</td>
</tr>
<tr>
<td>Mean</td>
<td>0.49</td>
<td>0.75</td>
</tr>
<tr>
<td>Range</td>
<td>0.00-20</td>
<td>20.00-35</td>
</tr>
<tr>
<td>BCS</td>
<td>3.10±0.22</td>
<td>2.0±0.22**</td>
</tr>
<tr>
<td><strong>Reproductive status (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal cyclic animals</td>
<td>90.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Bilateral smooth inactive ovaries</td>
<td>10.00</td>
<td>45.00</td>
</tr>
<tr>
<td>Delayed puberty</td>
<td>00.00</td>
<td>20.00</td>
</tr>
<tr>
<td>Endometritis</td>
<td>00.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Persistent corpus luteum</td>
<td>00.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Repeat breeding</td>
<td>00.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Mastitis</td>
<td>00.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Abortion</td>
<td>00.00</td>
<td>5.00</td>
</tr>
</tbody>
</table>

*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±SE)

<table>
<thead>
<tr>
<th>Oxidant/antioxidant markers</th>
<th>Low BLC group (NO = 10)*</th>
<th>High BLC group (NO = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondialdehyde (mmol mL⁻¹)</td>
<td>1.89±0.06</td>
<td>5.18±0.79***</td>
</tr>
<tr>
<td>Nitric oxide (μmol L⁻¹)</td>
<td>16.51±0.94</td>
<td>29.54±2.13***</td>
</tr>
<tr>
<td>Total antioxidant activity (mmol L⁻¹)</td>
<td>1.39±0.08</td>
<td>0.64±0.04***</td>
</tr>
<tr>
<td>Superoxide dismutase (μL⁻¹)</td>
<td>336.40±6.81</td>
<td>298.29±2.79***</td>
</tr>
<tr>
<td>Glutathione reduced (mmol L⁻¹)</td>
<td>6.98±0.23</td>
<td>1.59±0.19***</td>
</tr>
<tr>
<td>Selenium (μg L⁻¹)</td>
<td>135.36±0.39</td>
<td>118.90±2.04***</td>
</tr>
</tbody>
</table>

*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 (%)

<table>
<thead>
<tr>
<th>Parasitic infestation</th>
<th>Low BLC group (NO = 10)</th>
<th>High BLC group (NO = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coccidia</td>
<td>10.00</td>
<td>20.00</td>
</tr>
<tr>
<td>Fasciolus</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Babesia</td>
<td>0.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Mange</td>
<td>16.00</td>
<td>20.00</td>
</tr>
</tbody>
</table>

*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 industrialized 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 Staccop, 1995) and in turn 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 (Taupenot 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 17β (Dearth et al., 2002; Pine et al., 2006).

In this study, the highest BLC was found in animals suffering fromrepeat 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 (Gorbil et al., 2002). In the same time, adverse affects in all items of reproduction including conception, implantation of fertilized ovum, 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 Frapte 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 Hana 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-Amino-levulinic 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 (Ville 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 (Brun et al., 1995) and humeral (Hoffman et al., 1995) immunity. The condition is intensified by lead-induced oxidative stress (Erul et al., 2000). This immune suppression resulted in increase parasitic infestation by the different recorded parasites (coccidias, trichostongylus, 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.

REFERENCES


67


