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STUDIES ON ENVIRONMENTAL POLLUTION WITH LEAD ON SOME BLOOD SERUM BIOCHEMICAL CHANGES AND BLOOD PICTURE IN DRAUGHT HORSES IN ASSIUT GOVERNORATE

M. N. Abd-El-Salam*, A. A. Ali** and Z. M. Zaky***

*Dept. of Animal Med. Fac. of Vet. Med., Assiut University, **Animal Health Rresearch Institute. Assiut Laboratory and ***Dept. of Forensic Med. & Tox., Fac. of Vet. Med., Assiut University

ABSTRACT : Summary

Looking for causes of poor performance, wasting and earlier senility and deaths than expected, of working horses in our area was the main goals of the present study. Accordingly blood, neck hair, fecal and serum samples were collected from 40 horses aging from 7-15 years old. Of them 28 carrying cars (Hantor Cars) horses working in the streets of Aboutige and Assiut cities. The other 12 horses are kept on pasture at the villages of Assiut and Aboutige cities far away from highways, where they are not exposed to environmental pollution with lead. These appeared apparently clinically healthy and were used as control. Clinical examination of all animals in the investigation was done. Lead was estimated in the blood, neck hair and fecal samples of all examined animals by atomic absorption spectrophotometer, while serum samples were bio-chemically analyzed by spectrophotometer using chemical kits for some blood parameters such as glucose, urea, creatinine, total protein, albumin, globulin & A/G ratio, ALT, AST, AP and CK. Heparinised blood samples were used for haemogram using automatic blood cell counter. Our results revealed that the working horses showed poor performance, wasting, dullness, anemia and poor general condition with slightly increased shallow, difficult breathing and slightly increased, weak heart rats (32- 40 and 54 – 64/min) respectively. There was a significant ($p < 0.01$) higher content in lead (ppm) in the samples collected from working horses than that of control group. Microcytic hypochromic anemia was found in the working horses group. There was a significant ($P < 0.01$) decrease in serum glucose level associated with significant ($P < 0.01$) increase in blood urea and creatinine in working horses than that of the control group. A hypo-albuminemia and hyperglobinemia were also evident in the lead-exposed horses. No significant changes were found in serum TP and ALT, although there was a significant ($P < 0.01$) increase in the levels of serum AST, AP and CK in the working horses than the control. The lead pollution from automobile exhausts in Assiut and Aboutige cities is high and the long term exposure of the working horses to this pollution has a harmful effect on the liver, kidneys and adverse effect on the processes of erythrogenesis and utilization of iron stores of the body, which in turn is responsible for their poor performance, wasting, poor body condition, earlier senility and deaths than expected.

INTRODUCTION :

Lead (Pb) is a common cause of poisoning of domestic animals throughout the world. Nowadays considerable amounts of Pb have been mobilized into the environment. The lead derived from petrol additives contributes not only to the intake through inhalations but also to the intake through ingestion as a result of fallout from vehicle exhaustion nearly food crops (WHO, 1972). Organic lead used as a gasoline additives is the current chief source of environmental lead pollution (Waldron and Stofeny 1974). Environmental pollution with lead is a common assurance in cities and near major highways, where pasture is contaminated by exhaust fumes of automobiles. In some locations near lead smelters, lead poisoning is considered to be a predictable occurrence in horses. High lead levels have been reported in the atmospheres, soil and vegetation (Aronson, 1978, Burrows et al., 1981 and Burrows and Borchard 1982). Lead is considered one of the most important environmental pollutant introduced into the environment through the soil, air or water sources. It is absorbed from the alimentary tract as acetate or carbonate and its blood level provides a reliable indication of the lead status of the animal (Antonioua et al., 1989). Lead is one of the commonest causes of poisoning in farm animals particularly cattle, sheep and horses (Blood and Radostits 1989). The authors added that horses appear to be more susceptible to lead poisoning from grazing on the same pastures contaminated with lead than cattle. Young horses are particularly more susceptible than older horses and cattle grazing on the same pasture. In term of tons of anthropogenic material emitted annually, five major air pollutants account for 98% of pollution: carbon monoxide (52%), sulfer oxides (14%),, volatile organic compounds (14%), particular matter (4%) and nitrogen oxides (14%). The remainder consists of lead, which is down 90% since 1983, when it was banned from gasoline, and a myriad of other compounds considered under the category of hazardous air pollutants (Raymond et al., 1995). The authors added that for any individual locality this emission picture can vary widely. In the vicinity of a smelter, for example, sulfer oxides, metals and/or particulate matter dominate the pollutant profile, while in suburban areas where the automobile is the main source of pollution, carbon monoxide, volatile organic compounds and nitrogen oxides predominate. Environmental air pollution with Pb and Cd in Assiut Governorate was reported (Sharkawy and Rateb 2000). Looking for causes of poor performance, wasting and earlier senility and deaths than expected, of working horses in our area was the main goals of the present study.

MATERIALS AND METHODS :

Blood, neck hair, fecal and serum samples were collected from 40 horses aging from 7-15 years old. Of them 28 carrying cars (Hantor Cars) horses (Figure 1) from Aboutige and Assiut cities (Egypt). The other 12 horses (Figure 2) are kept on pasture at the villages of Assiut and Aboutige cities far away from highways, where they are not exposed to the environmental pollution with lead. These appeared apparently clinically healthy and were used as control. Clinical examination of all animals in the investigation was done (Kelly, 1974). Lead concentrations in blood, hair and feces were determined by atomic absorption spectrophotometer (G B C 906 Atomic Absorption). Samples were prepared for atomic absorption assay by a nitric acid modification of a previously described precipitation method (Slavin and Sprague 1964). Heparinized blood samples were used for haemogram using

automatic blood cell counter (Cell dyne 1700, Abott Company)). While serum levels of glucose, urea, creatinine, total protein, albumin, ALT,AST, AP and CK. were estimated spectrophotometry (Cecil CE292, Digital UV spectrophotometer) using chemical kits supplied by Boehringer Mannheim (Germany) after the methods of Trinder (1969); Chaney and Marbach (1963); Sheling and Wust (1969); Weichselbaum (1946); Doumas, et al (1971); Reitman and Frankel (1957); Belifield and Goldberg (1971) and Mercer (1974) respectively

Statistical analysis was done (Kalton, 1967) using Student's "t" Test to calculate the significance between control and working horses group. Probability values 0.05 and 0.01 were considered statistically significant.

RESULTS :

Clinical examination of the investigated horses illustrates the healthy condition of the control group, while working horses showed poor performance, wasting, dullness, anemic mucous membrane and poor body condition (Figure 3) with slightly increased shallow, difficult respiratory and slightly increased, weak heart rats (32-40 and 54–64/min) respectively. There was a significant ($p<0.01$) increase in content of lead (ppm) in the samples collected from working horses than that kept far away from highways (Table 1 and Figure 4). The variance in lead levels between the two groups were higher in blood followed by hair then feces. Examination of heparinized blood samples revealed a microcytic hypochromic anemia in the working horses group (Table 2). Biochemical serum analysis indicate significant ($p<0.01$) decrease in serum glucose and significant ($p< 0.01$) increase in urea, creatinine, AST, AP and CK in working horses than control one (Table 3). Hypoalbumineamia and hyperglobineamia associated with significant ($p<0.01$) decrease in A/G ratio were also recorded in working horses group.

This investigation could revealed that the second group (working group) is lead exposed through their exposure to the surrounding environment.

Table (1): lead levels (ppm) in blood, fecal and hair samples.

		Working horses (n=28)	Control horses (n=12)
Blood	Range	0.39–0.71	0.075 - 0.13
	Mean ±S.D	0.65 ± 0.04**	0.115 ± 0.025
Feces	Range	27.5 – 78.6	7.5 - 38.7
	Mean ± S.D	46.5 ± 7.8**	30.2 ± 6.9
Hair	Range	11.5 – 33.8	5.5 - 12.8
	Mean ± S.D	26.8 ± 5.6**	8.55 ± 2.39

**: P < 0.01.

Table (2): Haemogram# in lead-exposed and non-exposed horses.

Parameter	Working horses (n= 28)	Control horses (n=12)
RBC's ($x10^6$)	(3.47–6.78) 6.6 ± 1.35**	(6.89 – 9.23) 8.2 ± 0.78
HB (gm/dl)	(4.50 – 12.20) 9.1 ± 2.42**	(11.93 – 15 .17) 14.5 ±0.47
PCV%	(24.5 – 29.3) 28.2 ± 6.42 **	(34.97 – 41.85) 37.5 ± 1.75
MCV (fl.)	(37.7 – 47.5) 42.7 ± 2.52**	(41.37 – 47.79) 45.7 ± 0.72
MCH (pg.)	(12.43 – 16.72) 13.79 ± 0.53**	14.76 – 19.73 17.7 ± 1.26
MCHC (gm/dl)	(33.31 – 40.61) 32.3 ± 1.13 **	(36.34 – 42.95) 38.7 ± 0.92

Table (3): Serum biochemical analysis# of lead- exposed and non-exposed horses.

Parameter	Working horses (n= 28)	Control horses (n=12)
Glucose (mg/dl)	(35.6 – 62.0) 47.4 ± 7.49**	(45.7- 70.2) 57.6 ± 7.2
Urea (mg/dl)	(47.5 – 81.6) 57.6 ± 9.44 **	(20.0-40.8) 31.5 ± 6.44
Creatinine (mg/dl)	(0.98 – 1.6) 1.4 ± 0.30 **	(0.43 – 0.68) 0.52 ± 0.11
Total protein (gm/dl)	(5.37 – 7.74) 6.8 ± 091	(6.15-7.83) 7.3 ± 1.05
Albumin (gm/dl)	(1.51 – 3.28) 2.6 ± 0.55 **	(3.03 – 4.34) 3.8 ±0.43
Globulin (gm/dl)	(3.26 – 5.04) 4.2 ± 0.71**	(2.55- 4.15) 3.3 ± 0.52
A/G ratio	(0.36 – 1.81) 0.60 ± 0.20 **	(0.88 – 1.68) 1.2 ± 0.29
ALT (I.U./L)	(4.0 – 17.0) 6.7 ± 3.11	(4.0-21.0) 9.0 ± 5.96
AST (I.U./L)	(41.0 – 76.0) 57.9 ± 11.48 **	(19.0-52.0) 39.7 ± 11.84
AP (I.U./L)	(157.05–245.09) 198.3± 28.5* *	(90.88 –177.22) 146.3 ± 34.75
CK (I.U./L)	(8.10 – 32.38) 16.6 ± 8.96**	(8.10 – 16.19) 9.7 ± 3.41

#: Values represent range and mean ± SD
**: P < 0.01

Figure (1): Carrying car (Hantor car) working horse.

Figure (2): Horse kept at field a way from high ways (control group).

Figure (3): Working horse shows poor body condition, wasting and dullness

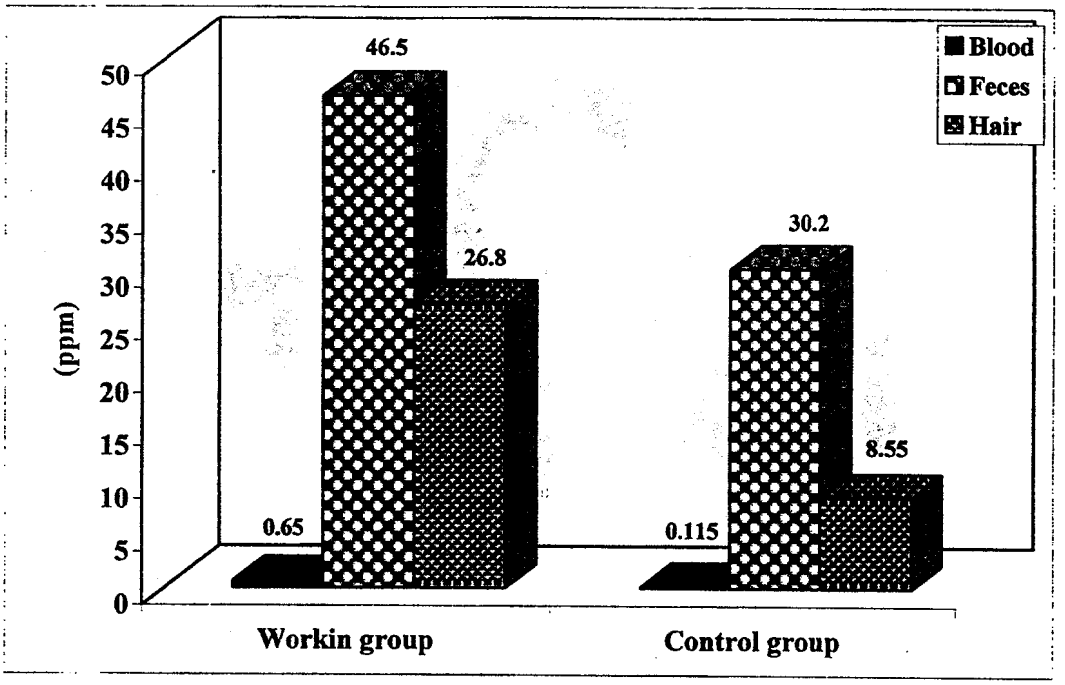

Figure (4): Lead levels (ppm) in blood, feces and hair.

DISCUSSION :

In the living animal with lead poisoning, levels of lead could be detected in blood, feces, hair, urine and milk. The estimation of blood levels is generally useful for determining the lead exposure of the animal and used most frequently to insure a clinical diagnosis of lead poisoning. Fecal level of lead represent unabsorbed and excreted lead deriving from the bones. Analysis for lead performed on blood and feces from the same animal can give some indication of the type of exposure involved. High values for the blood and feces indicate a relatively recent exposure, while high values in the blood but not in the feces indicates an exposure much further back in time (Allcrofit, 1951). In rural areas, levels of Pb in air of 0.1 $\mu g/m^3$ or less are found depending upon the degree of pollution due to urbanization, the amount of lead in city air range from 1-3 $\mu g/m^3$ and will occasionally be much higher under peak traffic conditions (WHO, 1972). It may be assumed that the intake of lead by inhalation in cities could on occasion be 100 µg/day. Environmental pollution can be monitored by analysis of blood, hair, feces and tissues (Mehennaoui et al., 1988). Background levels of blood lead in animals and man can give an idea in order to a certain the exact extent of environmental lead pollution specially in areas around lead mines and in cities with higher automobile densities (Adaudi et al., 1990). The authors added that background blood lead is the concentration of lead in whole blood resulted from the daily exposure to lead, which doses not produce, any clinical evidence of disease.

The clinical signs that observed on the working horses, in the form of poor performance, wasting, dullness, anemia, shallow, difficulty breathing and weak rapid heart sounds can illustrate a high level of exposure of these animals to environmental lead pollution from automobiles exhausts and other industrial emissions and reflect the harmful effects of the lead pollution on the general healthy condition of the exposed animals. Lead damages ATPase responsible for ATP production and utilization therefor it can interfere with oxidative metabolism (Mustafa et al., 1971). Blood lead level reflects the quantity of biologically active lead in the blood and it is a good estimate of current exposure (Vahter et al., 1991). Lead levels in blood greater than 0.3 ppm are usually considered indicative of toxicity and levels of 0.5 to 0.7 ppm have been associated with severe clinical signs (Kowalczky 1981). High blood and low fecal levels of Pb indicate that the lead was taken in some weeks previously but high blood and high fecal levels suggest recent ingestion and significant absorption (Blood and Radostits 1989).

Our results of lead level in blood (0.165 ± 0.025 ppm) of control horses (control) were higher than that suggested by Egan and O'Cuill (1970), who found that in horses normal figure of blood level of lead varies from 0.04 to 0.10 ppm. While our results of lead levels in the blood in working horses (0.65 ± 0.04) were higher than those obtained by Egan and O'Cuill (1970), who found that in horses with accumulative poisoning the blood level of lead was 0.28– 0.44 ppm, they were lower than that obtained by Sharkawy and Rateb (2000), who found lead level of 2.08 ± 0.169 ppm in blood samples of horses collected randomly from Assiut Governorate. The authors suggest that these higher levels of lead may return to contamination of feed stuffs and drinking water with Pb or/and pollution of the environmental air with Pb. Environmental pollution with heavy metals especially that of major health effects like Pb and Cd in Assiut Governorate was suggested also previously by Sharkawy and Rateb (2000).

Our results of lead level in hair in working horses (Table, 1) illustrate that hair represent a pathway of excretory mechanism of the lead and suggest a long term of exposure of these animals to high level of environmental lead pollution.

The microcytic hypochromic anemia (Table, 2) found in working horses indicates a high level of exposure and illustrates the adverse effect of lead poisoning on the process of erythrogenesis and the utilization of store iron of the body. Lead causes inhibition of the enzyme delta aminolevulenic acid dehydratase (ALA-D), resulting in failure of utilization of delta aminolevulenic acid which is excreted in increased quantities in the urine (Goyer and Rhyne 1973). Anemia in chronic lead poisoning is caused by tow basic defects: a shortened erythrocyte life span and impairment of hem synthesis (George and Duncan 1981). The authors added that lead causes increased concentration of protoporphyrin by inhibiting hem synthetase, the enzyme which combines protoporphyrin and iron to form hem. However, our results are not agree with that found by Sharkawy and Rateb (2000), who found no significant changes in RBC's and PCV values in horses blood collected randomly from Assiut Governorate, although environmental contamination with Pb and Cd has been suggested by the authors.

The alterations in the levels of serum estimated parameters such as significant ($p<0.01$) decrease in glucose, significant ($p<0.01$) increase in urea, creatinine, AST, AP and CK in

lead-exposed horses than non-exposed one (Table 3) can indicate exposure to a large amount of lead and explain the damage effect of lead on the kidneys and liver. However this can not be only return to the harmful effects of chronic lead poisoning, but also environmental contamination with cadmium, which has similar effects can be involved in the process resulting in additive effects. Moreover other illness can be also included, which resulting in increasing mobilization of lead from its deposits in the bones to a limit that exceed the binding capacity of the erythrocytes and serum albumin and passage of free, ionized lead to liver and kidneys and other tissues. Lead is known to affect the structure and function of various organs and tissues, including the brain, kidney and liver. In kidney, lead damages the proximal convoluted tubular cells and induces acid fast intra-nuclear inclusion Bodies (Goyer, 1991). Harmful levels of lead can be accumulated in tissues, after prolonged exposure to low quantities (Humpfreys, 1991). The author added that an increase of free or ionized lead only occurs when the binding capacity of erythrocytes and plasma protein is exceeded after exposure to a relative large amount of lead. Environmental lead contamination is often combined with cadmium, which has similar effects to those of lead (Radostits et al., 2000). Environmental contamination with Pb and Cd was reported in Assiut Governorate by Sharkawy and Rateb (2000).

The hypoalbumineamia in working horses than control one explained by the effect of lead on the albumin synthesis in the liver, while the hyperglobinaemia found in these animals may refer to persist stimulation of immune response by continuous exposure to lead pollution, which when prolonged leads to immune suppression and hypoglobinaemia, or may be compensatory to the hypoalbuminic status in these working horses. Lead poisoning has a toxic effect on sulfydryl-, carboxyl- and imidazole-containing protein and membrane protein (Fell, 1984). It can be concluded that our results revealed that lead pollution in Assiut and Aboutige cities is a fact. The long term exposure of the working horses to this lead pollution has a harmful effect on the liver, kidneys, adverse effect on the processes of erythrogenesis and utilization of iron stores of the body and interfere with oxidative metabolism, which in turn is responsible for their poor performance, wasting, poor body condition, earlier senility and deaths than expected. Therefor periodical monitoring of the level of lead in air pollution should be done and supplementation of the animals with antioxidant vitamins such as vitamin A, C and E, calcium source and high protein diet for working horses should be dominate to minimize the harmful effect of lead poisoning.

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دراسات على التلوث البيئى بالرصاص وما ينتج عنه من تغيرات فى المكونات البيوكيميائية والصورة الدموية لدم خيول الجر فى محافظة أسيوط

محمد نجيب عبد السلام*، عبد الرحمن أحمد على**، زكريا مختار زكى***

* قسم طب الحيوان – كلية الطب البيطرى – جامعة أسيوط ، ** معهد بحوث صحة الحيوان بأسيوط

*** قسم الطب الشرعى والسموم – كلية الطب البيطرى – جامعة أسيوط

تم إجراء هذا البحث على عدد ٤٠ من الخيول التي تتراوح أعمارها بين ٧-١٥ عام حيث تم جمع عينات لكل من دم ، شعر الرقبة ، روث ، ومصل من هذه الحيوانات حيث اشتملت الدراسة على مجموعتين الأولى عبارة عن عدد ٢٨ من خيول الجر (خيول جر عربات الحنطور) والتى تجول في مدينتى أبوتيج وأسيوط، والتى تتعرض يوميا للتلوث بالرصاص عن طريق استنشاق عادم السيارات بصورة مباشرة، وذلك لدراسة تأثير الرصاص على هذه الخيول، بينما كانت المجموعة الثانية عبارة عن عدد ١٢ من الخيول جمعت منها العينات، وكانت تعمل في مناطق بعيدة عن التلوث بعادم السيارات (فى حقول القرى التابعة لمدينتى أبوتيج وأسيوط حيث يستخدمها الفلاحون والمزارعون للذهاب بها إلى حقولهم وتستخدم أيضاً كزينة) والتي أظهرت الفحوص الإكلينيكية خلوها من أي أعراض مرضية وظهورها في صورة صحية جيدة، واعتبرت هذه المجموعة كضابط للبحث .

تم قياس مستوي الرصاص باستخدام جهاز الامتصاص الذري الطيفي لعينات الدم وشعر الرقبة، والروث بينما تم قياس مستوى الجليكوز واليوريا والكرياتينين والزلال الكلي والالبيومين والجلوبيولين وأنزيمات الالينين أمينوترانسفيرز والأسبرتيت أمينوترانسفيريز والالكالين فوسفاتيز، كما تم قياس عدد كرات الدم الحمراء والهيموجلوبين وحجم الخلايا المنضغط باستخدام عداد خلايا الدم الأوتوماتيكي.

أظهرت الفحوصات الإكلينيكية ظهور هبوط عام وإجهاد وأنيميا مع زيادة طفيفة فى التنفس مصحوبة بضيق في التنفس وزيادة مع ضعف في عدد ضربات القلب في خيول المجموعة الأولى بالمقارنة بالمجموعة الضابطة، كما أظهرت الفحوصات زيادة معنوية في مستوى الرصاص في عينات الدم وشعر الرقبة والروث في حيوانات المجموعة الأولى بالمقارنة بالمجموعة الضابطة كما أظهرت فحوصات الدم وجود نقص معنوى فى كرات الدم الحمراء والهيموجلوبين الأمر الذى أدى إلى ظهور أنيميا فى خيول المجموعة الأولى بالمقارنة بالمجموعة الضابطة. كما ثبت بالتحليل البيوكيميائى وجود نقص معنوي في مستوى الجليكوز والألبيومين مصحوبا بزيادة في مستوى اليوريا والكرياتينين والجلوبيولين وأنزيمات الأسبرتيت أمينوترانسفيريز والالكالين فوسفاتيز في المجموعة الأولى بالمقارنة بالمجموعة الضابطة. ويتضح من هذا البحث أن تعرض خيول الجر للرصاص الموجود بعادم السيارات لفترة طويلة يؤدي إلى التسمم المزمن بالرصاص الذي يعكس التأثير الضار على هذه الحيوانات.