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Influence of Leaded-Gasoline Regulations on the Blood Lead Concentrations in Murciano-Granadina Goats from Murcia Region, Southeast Spain

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Murcia (SE Spain) is a Spanish Autonomous Region located at the Southwestern Mediterranean Sea firstly devoted to farming activities and tourism trade. In recent years, these mentioned activities have required the improvement of the communication routes, mainly new highways. Also, Cartagena was known since antiquity for its mines, mainly of lead, and smelting plants were established in this zone. Vehicular lead emissions and fixed sources of lead such as foundries, petrochemical products and mines have been considered an important health hazard (Romieu et al. 1997). Also, several studies have shown that increased blood lead levels may cause behavioral problems or a decrease in the intelligence quotient (Grant and Davis 1990; CDC 1991; ATSDR 1993).

Murciano-Granadina goat is an adaptable Mediterranean breed originating from Murcia widely distributed in this Region with a total census of 90,000 goats (60 and 3% of the total goats in Murcia and Spain, respectively) (MAPA 2002). The population is distributed in farms with a mean of 65 goats per farm (Caballero and Buxadé 1996). In Spain, studies on heavy metals in slaughtered animals are scarce (López-Alonso et al. 2000) and lead levels has never been evaluated in this goat breed. Some results of an analytical survey on lead contamination in Murciano-Granadina goats in two distant periods of time, before and after leaded-gasoline regulations were applied in Spain, are shown in this paper. Three farms located in three areas with different environmental and antropogenic conditions were studied in order to evaluate the influence of the lead exposure from different sources and with special reference to the new leaded-gasoline regulations.

MATERIALS AND METHODS

Three different areas from Murcia Region (SE Spain), which have been selected for sampling according to the natural conditions and the antropogenic influence in respect to the lead. Blood samples were taken from a total of 137 Murciano-Granadina goats from three farms that belonged to these areas (Figure 1). The first area, located in La Union (Cartagena), is an ancient and important mining area dedicated to the extraction of Ag, Zn and mainly Pb. The mining activity was definitively closed down in 1991. Here we obtained samples from 23 and 21 goats in 1992 and 2001, respectively. Another area (Lorca) is devoted to intensive farming where the road traffic is very

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much intense. In this zone we selected a farm near to the most used highway. Here we obtained samples from 26 and 21 goats in 1992 and 2001, respectively. The last area was considered as a control. This area is located in Cagitán (Mula) a highland with extensive grazing of small ruminants (sheep and goat) and crops for dry farming and far from any urban, industrial or mining zones. Here we obtained samples from 26 goats in 1992 and 20 goats in 2001. In both periods the samples were collected in November.

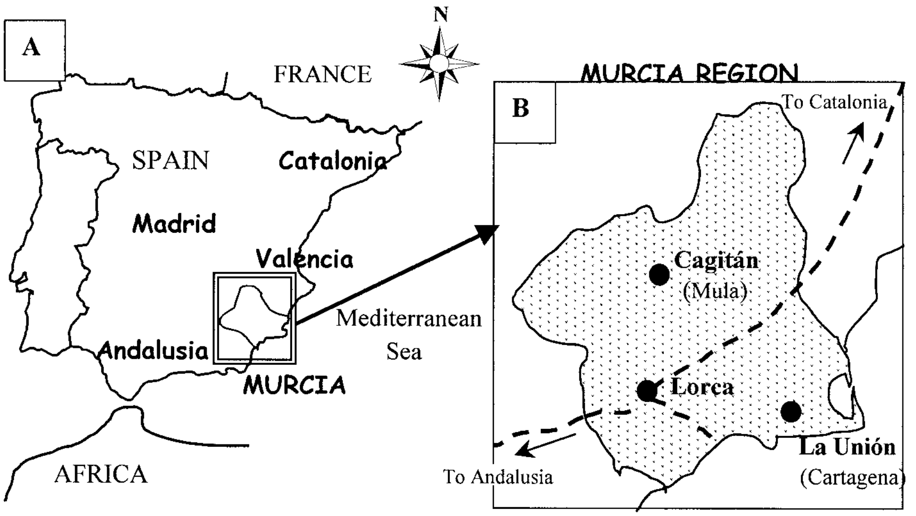

Figure 1. Maps showing the geographical location of Murcia Province, the areas studied and its influencing areas. *Scale A = 1:16,800,000.* Rural area = Cagitán (Mula); Mining area = La Unión (Cartagena); Highway area = Lorca.

Blood samples were obtained by punction into jugular vein using Venoject® needles and collected in heparinized tubes. Samples were refrigerated and immediately taken off to the Laboratory of Toxicology for analysis. Anodic Stripping Voltammetry (ASV) analysis method has been used to determine the concentrations of lead and cadmium. Our procedure has been previously published (García-Fernández et al. 1995). It consists of wet digestion of samples (0.5 ml of blood) using 0.5 ml of an acid mixture (nitric acid/perchloric acid/sulfuric acid, 8/8/1). The sample was then submitted to a progressive thermal treatment. A voltameter with a VA-646 processor and a VA-647 workstation (Metrohm, Switzerland) was used for analytical determination of lead. Lead standard solution (Sigma, St. Louis, Mo) has been used as reference and a detection limit of 5 µg/L in blood was obtained. The repeatability for the lead, determined by analyzing 10 identical samples of reconstituted lyophilized blood (European Union Reference Standards) CRM195, was 96.5 ± 1.2%. To calculate percentage recovery, we processed 10 blood samples, which had been spiked with known amounts of the lead analytical standards. The mean recoveries in blood was 102%.

Data were grouped by sampling areas and periods. They were expressed as mean ± standard deviation, standard error of the mean, minimum and maximum values. T tests for independent samples were used to examine differences both between sampling periods. To examine differences among areas of sampling we used One-way analysis of variance (ANOVA) test. All statements of significance were based on the 0.05 level of probability.

RESULTS AND DISCUSSION

Lead is a heavy metal widely extended and distributed in all ecosystems and is present in all living beings (García-Fernández et al. 1995; Santiago et al. 1998), in spite of this, no biological function is recognized in it (Goyer 2001). Although the study of heavy metals and its influence on the animal health is important, additional information about both environmental contamination and risk assessment can be available using animals as biomonitors (NRC 1991). The measurement of lead in blood is considered the best indicator of the recent exposure to this metal(NRC 1991, Garcia-Fernandez et al. 1997), an is thus an useful means of monitoring environmental contamination. This paper presents the results of an investigation on blood lead levels in goats as an useful tool to evaluate the environmental contamination and its evolution throughout the time in different areas of Murcia Region (Southeastern Spain).

Blood lead concentrations in goats from different areas and periods are presented in table 1. The total mean concentration of lead in blood from goats sampled in 1992 was slightly higher than in 2001 but the difference was not statistically significant (52.5 ± 3.1 μg/L in 1992; 45.6 ± 3.5 μg/L in 2001) (Table 1). However, the comparisons of the mean total concentrations among sampling areas showed significant differences: *Mining* > *Highway* > *Rural*.

Table 1. Lead in blood (μg/L) of Murciano-Granadina goats sampled in three farms from different areas in 1992 and 2001.

Year	Area	N	Mean	S.D.	S.E.M.	Min.	Max.	Median	G.M.
1992	Rural	26	20.4	7.5	1.5	13	42	18	19.4
	Highway	26	59.6*	11.9	2.3	28	87	58	58.4
	Mining	23	80.7	11.1	2.3	58	114	80	80.0
2001	Rural	20	17.3	8.4	1.9	7	40	16	15.6
	Highway	21	39.5*	10.7	2.3	22	68	38	38.1
	Mining	21	78.5	12.4	2.7	55	121	77	77.6

*Statistical difference at level 0.05.

N, number of samples; S.D., standard deviation; S.E.M., standard error of the mean; Min., minimum; Max., maximum; G.M., geometric mean.

These results confirm the well-known close relationship between human activities and lead exposure. On the other hand, taking into account both factors (sampling years and areas), only *Highway area* (Lorca) showed significant difference between years (Table 1). In the other zones (*Mining* and *Rural* areas) the lead levels in both sampling periods were similar.

Several environmental anthropogenic sources of this metal are known, however mining and lead-fuel combustion are considered as the most important sources of lead in mammals. In general, the presence of heavy metals in animals is firstly due to the ingestion of contaminated food where the heavy metals arrive by atmospheric deposition or biologically incorporated through the trophic chain (Garcia-Fernandez et al. 1995; Goyer 2001).

The most relevant finding was the difference found in the *highway area* where the mean blood lead concentration in 1992 was 1.5-fold higher than in 2001 (Table 1), being this difference statistically significant. This zone has not any direct and important source of lead, except the combustion of the leaded-gasoline by vehicles. The road traffic in this zone is high due to the intense agricultural activity and to the passage of tourists towards the littoral areas from Murcia, Andalusia (South Spain) and West Mediterranean coasts (Valencia and Catalonia) (Figure 1B). The Daily Mean Intensity (DMI) of cars in the main highway of this zone was about 38,000 in 2000. Since 1998 several regulations were approved in the European Union (EU) and Spain restricting progressively and finally banning the use of leaded-gasoline in vehicles (Directive 98/79/CE; Royal Decree 785/2001). The total prohibition of leaded-gasoline in Spain was effective since August 1, 2001. The samples of the second period were collected in November 2001. Also, Spanish Government established another law subsidizing the renovation of the total number of old cars in order to reduce emissions of lead and sulfur (Law 39/1997). These regulations may explain the significant decreasing of the blood lead concentrations observed in goats. According with Romieu et al. (1992, 1997) residents in areas of intense vehicular traffic have blood lead concentrations much higher than populations exposed to less vehicular traffic.

The statistical significance of the mean comparisons between sampling years failed in the *mining* and *rural* areas (Table 1). In the last fifty years, the human activity in the *rural area* has not suffered notable modifications and may explain why no differences in blood lead of the goats from this area were found. Moreover, this area have not important roads and the farming vehicles use diesel-fuel. In respect to the *mining area* near Cartagena we must note that mining and smelting activities were closed-down in 1991, a year before the first group of samples were collected. Many attempts to recovery the zone have been initiated by the Autonomous Government. However, this zone is filled with mineral residue deposits originated form the mining process. The wind and rainwater mobilize deposited metals and they are incorporated to the atmosphere, soil and waters of the zone. These facts would explain the highest blood lead concentrations in both periods of time with respect to the other zones.

In conclusion, the governmental regulations restricting the use of leaded-gasoline since 1998 and the subsidized renewal of the old cars initiated in 1997 in Spain have improved the environmental contamination in respect to the lead in those areas influenced by intense road traffic included urban areas. In mining zone, the similar results in both periods of time (1992-2001) suggest that the leaded-gasoline restrictions applied by Spanish and European laws have not improved the environmental health status of this highly contaminated zone yet.

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REFERENCES

ATSDR (1993) Lead. TP-92/12. Agency for Toxic Substances and Disease Registry. Atlanta, G.A.

Caballero JR, Buxadé C (1996) El subsector caprino en España. In Buxadé C (ed) Zootecnia. Bases de producción animal. Mundi-Prensa, Madrid, p 31

CDC (1991) Preventing lead poisoning in young children. A statement by the Centers for Diseases Control. Atlanta, GA, Centers for Disease Control and Prevention

Directiva 98/79/CE del Parlamento Europeo y del Consejo de 13 de octubre de 1998 relativa a la calidad de la gasolina y el gasóleo y por la que se modifica la Directiva 93/12/CEE del Consejo. Diario Oficial Europeo L350: 58-68

Garcia-Fernandez AJ, Motas-Guzmán M, Navas I, María-Mojica P, Luna A, Sanchez-Garcia JA (1997) Environmental exposure and distribution of lead in four species of raptors in Southeastern Spain. Arch Environ Contam Toxicol 33: 76-82

Garcia-Fernandez AJ, Motas-Guzmán M, Navas I, María-Mojica P, Romero D (1999) Sunflower meal as cause of chronic copper poisoning in lambs in Southeastern Spain. Canadian Vet J 40: 799-801

Garcia-Fernandez AJ, Sánchez-García JA, Jiménez P, Luna A (1995) Lead and cadmium in wild birds in Southeastern Spain. Environ Toxicol Chem 14:2049-2058

Grant LD, Davis JM (1990) Effects of low-level lead exposure on pediatric neurobehavioral and physical development: current findings and future directions. In Smith M, Grant LD, Sors A (eds) Lead exposure and child development: an international assessment. MT Press, Lancaster, p 49

Goyer RA (2001) Toxic effects of metals. In Klaassen CD (ed) Cassarett and Doull's Toxicology. The basic science of poisons, 6th edition. McGraw-Hill New York

Law 39/1997 (October 8, 1997) Por la que se aprueba el programa PREVER para la modernización del parque de vehículos automóviles, el incremento de la seguridad vial y la defensa y protección del medio ambiente. Spanish Official Bulletin, Madrid

López-Alonso M, Benedito JL, Miranda M, , Castillo C, Hernández J, Shore RF (2000) Arsenic, cadmium, lead, copper and zinc in cattle from Galicia, NW Spain. Sci Total Environ 246: 237-248

MAPA-Ministerio de Agricultura, Pesca y Alimentación (2002) Censos y Producciones Ganaderas. In Hechos y Cifras del sector agroalimentario y del medio rural español, 6ª ed. Ed. Secretaría General Técnica, MAPA, Madrid, p 42-46

NRC-National Research Council (1991) Animals as sentinels of environmental health hazards. National Academic Press, Washington, D.C.

Romieu I, Lacasana M, McConnell R and the Lead Research Group of the Pan-American Health Organization (1997) Lead Exposure in Latin America and

the Caribbean. Environ Health Perspect 105: 398-405
Romieu I, Palazuelos E, Meneses F, Hernández M (1992) Vehicular traffic as a determinant of blood-lead levels in children: a pilot study in México City. Arch Environ Health 47: 246-249
Royal Decree 785/2001 (July 6, 2001) Por el que se adelanta la prohibición de comercialización de las gasolinas con plomo y se establecen las especificaciones de las gasolinas que sustituirán a aquéllas. Spanish Official Bulletin, Madrid, 162: 24775-24776
Santiago D, Motas-Guzmán M, Reja A, María-Mojica P, Rodero B, Garcia-Fernandez AJ (1998) Lead and cadmium in red deer and wild boar from Sierra Morena (Andalusia). Bull Environ Contam Toxicol 61: 730-737