Epidemiologic Study of an Outbreak of Clenbuterol Poisoning in Catalonia, Spain

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Synopsis

In an investigation of 113 cases of clenbuterol poisoning in Catalonia, Spain, in 1992, more than 50 percent of those affected were found to have had symptoms of nervousness, tachycardia, muscle tremors, myalgia, and headache. There was no significant difference in the distribution of symptoms according to sex (P = 0.97). The period of incubation varied between 15 minutes and 6 hours and the duration of symptoms between 90 minutes and 6 days. Clenbuterol was detected in 47 urine samples in amounts ranging from 11 to 486 parts per billion. No traces of clenbuterol were found in serum samples.

Intoxication occurred in association with the ingestion of veal liver, irrespective of the way in which the liver had been cooked. The association between consuming liver and falling ill was statistically significant (P < 0.0001). In one family, the suspected source of intoxication was meat (veal tongue) and in another canneloni. None of the patients died as a result of the intoxication.

The findings reinforce the need to uphold the prohibition of the use of clenbuterol in cattle farming in those countries and communities where it already exists and to contemplate a stricter regulation of its therapeutic use.

CLENBUTEROL, a β-agonist drug with affinity for β_1 and β_2 receptors, is licensed as a bronchodilator for use in human medicine in Spain and other countries in the European Community (*1–3*). It is also used as bronchodilator and tocolytic for the treatment of respiratory disease in horses and cattle and to relax the uterus in cows at parturition (*4–6*).

The anabolic effects of clenbuterol on muscle mass and body fat, however, have been exploited illicitly in meat production (*7,8*). This agent causes regression in body lipids, muscle growth, and weight gain and has been illegally used in the past as a growth promoter ('repartition factor') in young cattle (*9–12*). It accumulates in the liver and, to a lesser degree, in muscle. Although the Commission of the European Community banned the use of this drug in animal feeding (*13*), the benefits to be obtained by increasing the ratio of lean to fat in the carcass by dosing the animals with black market supplies of β-agonists must be tempting, since there have been a number of outbreaks of clenbuterol-related poisoning in the last 3 years in Europe.

In 1990, there was an outbreak in France in which 22 people were affected as a result of the consumption of clenbuterol residues in veal liver (*14*). In the same year, a total of 135 cases were reported in another outbreak that occurred in several autonomous regions in Spain. Veal liver was likewise the source of intoxication (*15-17*). During the first 3 months of 1992, a further episode of clenbuterol poisoning was registered in Spain. Of a total number of 232 cases, 113 occurred in Catalonia (an autonomous region in northeast Spain with a population of 6 million).

The aim of this study is to present the results of investigating the clenbuterol poisoning outbreak in Catalonia.

Materials and Methods

On January 28, 1992, the Department of Health and Social Security in Barcelona (Delegación Territorial de Sanidad de Barcelona) was notified that four patients had attended the emergency rooms of two hospitals in the town of Sabadell presenting symptoms of tachycardia and muscle tremors. All four patients had eaten the same food at the same restaurant in Sabadell the day before. The menu included soup, spaghetti with tomato sauce, and grilled veal liver.

Epidemiologists from the health department in Barcelona contacted the health authorities in Sabadell and requested that urine and blood samples be obtained from the patients affected. An investigation was made to determine the presence of other cases with similar symptoms in all other hospitals in the town. The same request was made to the health authorities in Terrassa, a town close to Sabadell that shares similar health care resources.

An epidemiologic questionnaire was drawn up for each patient to register personal data such as name, surname, place of residence, clinical symptoms, day and time at which symptoms developed, food consumed during the day symptoms appeared, and establishments where liver and other bovine products—the suspected sources of food poisoning—were consumed. Patients also were asked if they were under medication with adrenergic agonists or antagonists. This epidemiologic inquiry also was conducted for controls chosen from the same families in which the cases occurred.

A copy of the epidemiologic inquiry form was sent to all hospital and primary care emergency services in the area together with a case definition (*16*) developed by the Ministry of Health of Spain as a result of the outbreak of clenbuterol poisoning that had occurred in 1990. The definition read "appearance of characteristic clinical manifestations subsequent to ingestion of liver, with abrupt onset after a latency period of between 30 minutes and 6 hours. The clinical profile is characterized by easily observable muscle tremors and palpitations-tachycardia, frequently accompanied by nervousness, cephalalgia, and myalgia, lasting for about 40 hours."

Instructions also were given for collected urine and serum samples to be sent to the Catalan Department of Agriculture Laboratory (Laboratori Agrari del Departament d'Agricultura, Ramaderia i Pesca de la Generalitat de Catalunya).

Based on data obtained from patients and restaurant proprietors as to where the potentially contaminated food was procured, local veterinary authorities and personnel from the Department of Health and Social Security examined official documents pertaining to the origin of the foodstuffs and authorizing their transportation. They also collected samples of leftover food, eaten at home by those affected, for analysis in the Department of Agriculture Laboratory.

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Samples of serum and urine of the patients and samples of the associated food were assayed for the presence of clenbuterol using high-performance liquid chromatography (HPLC) (*18,19*). The samples were purified with extraction column in solid phase (Chem Elut) of diatomees soil sediment washed with n-hexane. Subsequently the clenbuterol was extracted by adding hydrochloric acid 0.01 N. The extract was analysed by HPLC in an inverse phase using a diode array detector.

Samples of associated food were stored and transported frozen at minus 20° C. Samples of serum and urine of the patients were stored and transported at 4° C.

In all cases, the delay between the time the sample was collected and the analysis was done was not more than 48 hours. For all kinds of samples a test was considered positive when clenbuterol at concentrations of 5 parts or more per billion (ppb) was identified. This was the minimum level that could be detected by the laboratory.

Study data were analyzed by the SPSS and Epi-Info statistical software packages. The chi-square test and the Fisher's exact test were used for the comparison of proportions. Means were compared using the Student's t-test. Statistical significance was taken as a less than 5 percent likelihood of occurring by chance.

Results

A total of 113 cases met the definition. We conducted a epidemiologic inquiry into these 113 cases and 37 controls by direct face to face interview or by telephone. Among the 113 cases, there were 52

Table 1. Distribution of 113 cases of clenbuterol poisoning in Catalonia, Spain, 1992, according to age and sex

Age (years)	Females	Males	Total
0–10	2	1	3
11–20	5	10	15
21–30	9	12	21
31–40	14	18	32
41–50	12	12	24
51–60	9	5	14
Older than 60	1	3	4
Total	[1]52	[2]61	113

[1]46 percent. [2]54 percent.

Table 2. Distribution of symptoms in 113 cases of clenbuterol poisoning in Catalonia, Spain, 1992

	Yes		No	
Symptoms	Number	Percent	Number	Percent
Tachycardia	106	93.8	7	6.2
Muscle tremor	106	93.8	7	6.2
Nervousness	103	91.2	10	8.8
Headache	78	69.0	35	31
Myalgia	73	64.6	40	35.4
Retro-ocular pain	36	31.8	77	68.2
Vomiting	26	23.0	87	77.0
Asthenia	23	20.4	90	79.6
Nausea	14	12.4	99	87.6

Table 3. Concentration of clenbuterol in nine samples of veal liver taken during 1992 outbreak of poisoning in Catalonia, Spain

Sample number	Parts per billion
1	1,980
2	640
3	50
4	5,395
5	26
6	88
7	23
8	3,600
9	19

women (46 percent) and 61 men (64 percent), ages 8 to 73 years (mean age 37.15 ± 13.71 years). There was no statistically significant difference between the mean age of women (38.02 ± 12.86 years) and men (36.15 ± 14.31 years) ($P = 0.2$). The distribution of cases according to age and sex is shown in table 1.

More than half of those afflicted presented symptoms of tachycardia, muscle tremors, nervousness, myalgia, and headache (table 2). There was no significant difference in the distribution of symptoms according to sex ($P = 0.97$). The interval between exposure and onset ranged from 15 minutes to 6 hours (fig. 1). The duration of symptoms varied between 90 minutes and 6 days. None of the patients were under medication with adrenergic agonists or antagonists.

Of the 113 people affected, 91 went to hospital emergency rooms. Six were subsequently admitted to the hospital, three for observation, two for the treatment of tachycardia, and one for the treatment of suspected carbon monoxide poisoning. All hospitalized patients recovered and were discharged a few hours after admission. None of the patients died.

Urine samples were collected from 45 patients and both urine and serum samples from another 2 patients. So, we were able to analyze 47 samples of urine and 2 serum samples. Clenbuterol was detected in the 47 urine samples in amounts ranging from 11 to 486 ppb. No traces of clenbuterol were found in the two serum samples. Onset of symptoms for most cases occurred between January 25 and 29 (fig. 2).

A study of the circumstances showed that intoxication occurred in 52 people from 32 families, 33 in connection with one restaurant, and 28 from a company canteen. Epidemiologic study revealed that in the restaurant, the canteen, and in 30 of the 32 families affected, intoxication occurred in association with the ingestion of veal liver, irrespective of the way in which the liver had been cooked. The rate of attack among exposed subjects was variable (up to 97 percent in the canteen).

Comparing cases and controls, the association between consuming liver and falling ill was statistically significant ($P < 0.0001$). In one family, the suspected source of intoxication was veal tongue and in another canneloni, although it was not possible to determine the precise ingredients used to fill the canneloni. It is probable that liver was used because it is a usual practice in the preparation of canneloni. Moreover, we knew that the meat and the kidneys of the animals involved were sent to other regions of Spain.

With regard to the suspected source of contamination, 16 samples of veal liver were analyzed, and clenbuterol was found to be present in 9 of these in amounts ranging from 19 to 5,395 ppb (table 3). Three samples of meat obtained from establishments where the affected people had purchased foodstuffs proved negative. None of these samples were from leftover meals eaten by affected subjects.

Further investigation showed that in all cases the liver ingested came from animals that had been slaughtered in a slaughterhouse in Sabadell on January 23 and 24 and had been delivered to all the establishments in question by the same distributor.

Discussion

The clinical and epidemiologic characteristics of the cases studied, the epidemic time curve, and the high levels of clenbuterol observed in blood samples are relevant findings. Both the case definition used and the presence of high concentrations of clenbuterol in positive samples were of particular value in the diagnosis, since the characteristically short incubation period and clinical symptoms of muscle tremors, tachycardia, nervousness, headache, and myalgia were observed in almost all cases. Other chemicals were not assayed because clenbuterol was the only β-agonist employed in the feeding of animals in Catalonia.

Because HPLC is a reference method for confirmation and quantification of clenbuterol recognized by the European Community and because all the results were clear, no single result was confirmed by mass spectrometry. Besides, HPLC is an easier technique than mass sprectrometry (*19,20*).

With regard to the case definition used in our study, two aspects are of particular significance—the duration of symptoms and the fact that the onset of symptoms occurred after the ingestion of liver. It should be borne in mind that in some patients clinical manifestations have been known to last several days (up to 6). Consequently, the case definition should be modified to include the observation that symptoms may last several days.

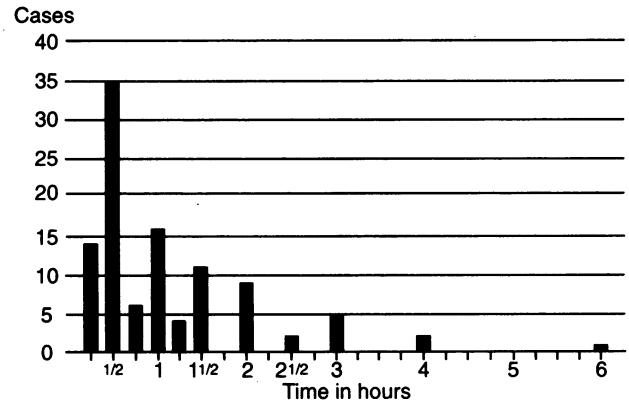
Likewise, the results of our study show that liver is not the only source of clenbuterol-related poisoning. In some of our patients, veal tongue and canneloni were the source of poisoning. Although urine samples were not obtained from these patients, it is highly likely, given the connection of these persons with others in whom the presence of clenbuterol was analytically confirmed, that the meat consumed came from the same animals whose livers were involved in the outbreak.

Since concentrations of clenbuterol observed in liver samples were very high, greater than 5,000 ppb, (traces amounting to 125–250 ppb are normally found after therapeutic dosing), it is probable that sufficient amounts of clenbuterol to cause intoxication may be present in tongue or other muscle mass of the involved animals, because clenbuterol, after being metabolized in the liver, passes to the blood and from the blood to the muscle mass.

Cinnamon used in locally made desserts was implicated in an earlier clenbuterol-related outbreak that occurred in Aragón, Spain, in 1991 affecting 59 persons. Accidental contamination of the cinnamon, obtained in an establishment where the proprietors were also cattle farmers, was postulated as the source of poisoning (*21*). In view of these data, it would be advisable to exclude the type of food consumed from the case definition of clenbuterol-related food poisoning.

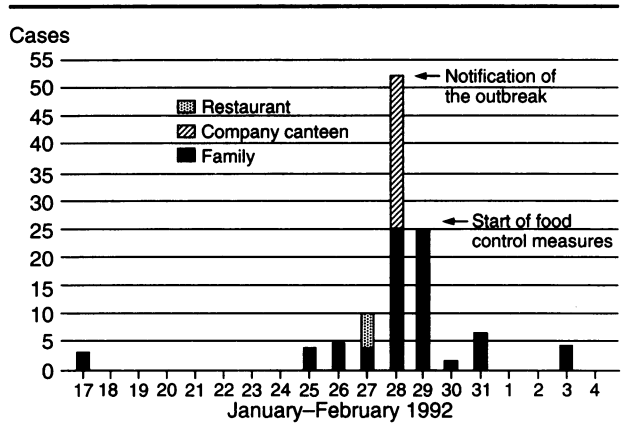
In relation to the epidemic time curve, it should be noted that three cases had already occurred by January 17, 11 days before the outbreak was reported to the health authorities. The delay with which these cases (without analytical confirmation) were reported, despite the fact that they conformed to the case definition of clenbuterol poisoning used, would indicate that probably they were not related to the animals raised by the same cattle farmers and slaughtered on January 23 and 24. In contrast, the four cases that occurred on February 3 are clearly associated with the latter, since it was possible to

Figure 1. Time elapsed between food consumption and the onset of clinical manifestations of clenbuterol poisoning of 105 persons[1] in Catalonia, Spain, 1992



[1]For 8 persons, the interval was unknown.

Figure 2. Epidemic time curve[1] of an outbreak of food poisoning following the consumption of clenbuterol–containing veal liver, Catalonia, Spain, 1992



[1]In 1 case, the day of onset was unknown.

show that the liver they consumed on that date was bought on January 29.

A rapid resolution of the outbreak occurred once health authorities had been notified and food quality control measures had been put into effect. In agreement with other authors (*14,22,23*), cooperation between epidemiologic and veterinary surveillance systems is considered to be of fundamental importance in the prevention of further cases of clenbuterol-related food poisoning.

In summary, the findings of this study reinforce the need to uphold the prohibition of the use of clenbuterol in cattle farming in those countries and communities where it is already fed to animals and to contemplate a stricter regulation of its therapeutic use.

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