Aldicarb toxicosis in a dairy herd

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In July 1988, 3 cows were found dead outside the milking parlor shortly after having been milked. The owner of the dairy did not pursue the cause of the problem at that time because no other cows appeared to have clinical signs of disease. The owner suspected that the cows might have been electrocuted. Additional problems on the farm were not experienced until September 1988, when 6 cows died during or shortly after morning milking. All cows had been clinically normal when entering the milking parlor and had produced acceptable quantitiies of milk. No additional cows were observed to be ill at that time. However, within 5 minutes after ingesting rumen contents from 1 of the dead cows, a dog became atactic, with diarrhea and muscular tremors. The dog was treated immediately with atropine sulfate and was clinically normal 4 hours later.

An investigation was conducted by the ambulatory section of the College of Veterinary Medicine to determine potential causes of the cattle deaths. The herd consisted of approximately 150 lactating cows, 45 nonlactating cows and heifers, and 60 calves. Death of all cows occurred on or approaching a weekend while the cows were in or shortly after they left the milking parlor. The syndrome appeared to be an "all or nothing" event; all affected cows died suddenly, and clinically affected cows were not observed.

Cows were brought in from pasture prior to milking and were kept in a holding pen for up to 3 hours with access to water, hay, salt blocks, and minerals. They were being fed a commercial 16% protein grain ration ad libitum while being milked and corn silage after milking. No other animals on the farm were given this commercial feed, which was delivered twice monthly. The particular batch of feed had been delivered 10 days before the episode commenced. Several other dairies in the area were using feed delivered from the same truck, but were not experiencing problems.

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The milking parlor was double-4 configuration, with gravity flow feeder pans supplied from a large bin overhead. Before and after milking, cows had access to large round bales of hay consisting mainly of moldy Johnson grass. Water sources consisted of 2 farm ponds and a concrete water tank in the holding pen, water to which was supplied by a well. The pasture where the cows grazed was of poor quality, principally fescue and orchard grasses overgrown with pigweed, cocklebur, nightshade, purple mint, and pokeweed. Other than 1 16-ounce can of an organophosphorus insecticide, no organophosphorus, carbamate, or chlorinated hydrocarbon insecticides were observed on the farm. Potential causes of the problem, considered at that time, included electrocution, grain overload, and several toxicoses, including organophosphorus insecticides, purple mint, cocklebur, blue-green algae, and nitrate.

Postmortem examination conducted on one of the cows revealed a small amount of interlobular pulmonary edema, but no other remarkable lesions. Histopathologic findings were limited to the lungs and consisted of bronchitis/bronchioloties characterized by mild epithelial hyperplasia and bronchiolar constriction. Also observed were a few areas of intraalveolar hemorrhage and mild intralobular edema. The cause of death was considered to be respiratory failure of unknown origin, but possibly attributable to anaphylaxis.

Organophosphorus insecticides were not detected in a representative sample of rumen contents obtained from 4 areas of the rumen. Brain acetylcholinesterase activity was 85% of the normal value when analyzed 2 days after death of the cow. The aqueous humor had negative reaction for nitrate. Rumen pH of 6 and sudden death tended to rule out grain overload-rumen acidosis. Microscopic examination of the pond water revealed no toxic blue-green algae. Purple mint toxicosis and cocklebur poisoning were ruled out on the basis of histologic evaluation of lung and liver tissue, respectively. The diagnosis considered at that time was pulmonary hypersensitivity type reaction, possibly to mold spores. Recommendations included eliminating the moldy feed from the diet, clipping

the pastures, and removal of the grain mixture from the overhead bin, followed by replacement with fresh feed.

Further problems were not observed until 6 weeks later, (November 1988) when 9 cows died while or shortly after being milked. However, in contrast to the previous 2 cases, 9 additional cows were observed to be ill, but did not die. The first, second, and fifth cows died eating from feeder No. 5, and another cow became ill. The second and sixth cows eating from feeder No. 7 and the fifth cow eating from feeder No. 4 also died. The exact feeding pan number used by the other dead or ill cows was unknown.

Clinical signs of toxicosis observed in the surviving affected cows included hypersalivation, abdominal bloating, lacrimation, fine muscular tremors, marked bradycardia (36 beats/min), respiratory distress, and transient diarrhea. Rectal temperature of 2 affected cows was 38.5 and 39.0 C, respiration was 38 and 46 breaths/min, and both cows had decreased ruminal motility. Postmortem examination of both cows revealed findings identical to those of the cow examined earlier.

Cows were currently being fed a complete 16% dairy ration with cottonseed while being milked, as well as corn silage and alfalfa hay after milking. This feed had been delivered 9 days previously, and again, neighboring dairies that used the same feed did not experience any problem. Little pasture grass existed at the time and the toxic plants previously mentioned had been killed by frost. Because the clinical syndrome resembled organophosphorus toxicosis, affected cows were treated with 0.5 mg of atropine sulfate/kg of body weight. Response was evident; all treated cows were clinically normal the following day.

A revised list of possible toxicoses consisted of organophosphorus, organochlorine, and carbamate insecticides, nitrate, urea, gossypol, mycotoxins, nitrous oxide, strychnine, and arsenic. Blood acetylcholinesterase determination for 1 of the clinically affected cows revealed 83% of the activity detected in a control sample. However, the test sample had been obtained approximately 4 hours after the syndrome commenced. Caudate nucleus acetylcholinesterase activity was 65% of the normal value, which was interpreted as being indicative of exposure to an acetylcholinesterase inhibitor, but not toxicosis.

Representative rumen content samples obtained from 4 areas of the rumen in 2 dead cows were chilled and transported to the Oklahoma Animal Disease Diagnostic Laboratory for insecticide analysis. Results of gas and thin-layer chromatography for organophosphorus, carbamate, and organochlorine insecticides were negative at detection limits of < 0.001% for carbamates and < 0.00001% for organochlorine and organophosphorus insecticides. However, owing to transport time, analyses were conducted 4 days after death of the 2 cows. Aqueous humor was again negative for nitrate reaction. The suspect feed did not contain urea, aflatoxin, zearalenone, or deoxynivalenol mycotoxins. The feed, however, contained 0.062% gossypol, an acceptable value for mature ruminants. Liver and kidney samples from 1 of the dead cows contained normal background values of lead and arsenic, and strychnine was not detected.

Two months later (January 1989), 5 additional cows died during or shortly after being milked. The first 4 cows that entered the milking parlor on the left side died soon after ingesting their feed. Several additional cows were allowed to eat from the feed containers on the left side of the milking parlor; all developed hypersalivation, dyspnea, and fine muscular tremors. Affected cows were administered atropine sulfate (0.5 mg/kg) immediately after onset of signs of toxicosis, and all were clinically normal 4 hours later.

Chilled rumen contents obtained from various areas of 1 of the dead cows did not induce clinical signs of toxicosis after ingestion by laboratory mice. Again, 4 days lapsed between death of the cow and ingestion of the rumen contents by mice. However, when mice ingested a random sample of suspect feed that was left in the feeders after the cows died, they had hypersalivation and muscular tremors; death ensued within 20 minutes. Two mice with the aforementioned signs of toxicosis responded to atropine sulfate and were clinically normal 2 hours later.

Extracts of the suspect feed were analyzed by use of thin-layer and high-pressure liquid chromatography. Results of thin-layer chromatography indicated presence of carbamate insecticide and those of high-pressure liquid chromatography confirmed the existance of 0.037% aldicarb, an extremely toxic carbamate. Analysis of the feed taken from the feed pans in question from the previous episode revealed 0.05% aldicarb. Analysis of feed from pans of unaffected cows as well as samples of feed from the feed bin did not reveal the presence of aldicarb.

Carbamate insecticides consist of cyclic or aliphatic derivatives of carbamic acid. Twenty compounds covering a wide range of toxicity currently exist. Some are considered only moderately toxic (ie, carbaryl, with oral LF_{50} in rats of 850 mg/kg), whereas others are considered extremely toxic (ie, aldicarb, with oral LD_{50} in rats of 0.8 mg/kg).[1] The mechanism of action of carbamates, like organophosphorus insecticides, is inhibition of acetylcholinesterase, and signs of poisoning are attributable to accumulation of acetylcholine and, thus, excess stimulation of the parasympathetic nervous system.[1,2] Typical signs of acetylcholinesterase inhibition include lacrimation, hypersalivation, miosis, muscular tremors, respiratory distress, diarrhea, and death.[1-4] Inactivation of acetylcholinesterase by carbamates involves a weaker and less stable binding than that with organophosphorus.[5] There-

fore, carbamates, are rapidly reversible inhibitors of acetylcholinesterase.[1,5] For this reason, carbamate-poisoned animals respond well to atropine sulfate (0.5 mg/kg, repeated as needed at 3- to 4-hour intervals), which acts to block the parasympathetic effects of excess acetylcholine. The drug 2-pyridine aldoxime methiodide (2-PAM), commonly used to liberate acetylcholinesterase in animals or people with organophosphorus poisoning, is ineffective for carbamate toxicosis. The rapid reversal of carbamate-inhibited acetylcholinesterase precludes the need to add another short-acting, although weak, inhibitor of acetylcholinesterase such as 2-PAM, because it would merely potentiate the deleterious effects of the carbamate.[1,3]

The use of organophosphorus and carbamate insecticides in agriculture, households, and home gardens has markedly increased over the last several years because of government restrictions on the more persistent, although often less toxic, chlorinated hydrocarbon insecticides.[6] Along with increased use of organophosphorus and carbamate insecticides has come a corresponding increase in livestock and companion animal poisonings.[6] Most carbamate poisonings involve commonly used products such as carbaryl and carbofuran. Aldicarb, unlike many carbamates, is extremely toxic via oral and dermal routes.[1] For this reason, it is not commonly used and is seldom involved in livestock or companion animal poisonings. The product is commercially available only as 10 and 15% granules because of the extreme toxicity of the parent compound.[7] In some states, aldicarb is acceptable for use in controlling certain insects, mites, and nematodes on citrus, cotton, sugar beets, potatoes, peanuts, ornamentals, pecans, sorghum, soybeans, and sugarcane; however, it is to be used only as a soil application.[7] According to the owner, aldicarb had never been used on this farm.

The literature contains numerous reports of organophosphorus and organochlorine toxicoses in cattle and other domestic animals. The clinical effects of toxic concentrations of organophosphorus compounds often persist owing to chronic acetylcholinesterase inhibition. Prolonged inhibition of acetylcholinesterase makes diagnosis relatively easy by determination of free acetylcholinesterase activity. Also, many of these organophosphorus compounds, or at least their metabolites, may persist in rumen contents or in the animal's system for several hours or days, which increases the chances of their detection. Carbamate compounds, however, induce toxic effects that may last only a few hours, leaving little evidence of their presence in dead animals and often no clinical signs of toxicosis in surviving animals. The short action of carbamates is attributable to their rapid dissociation from acetylcholinesterase, leaving free enzyme to hydrolyze acetylcholine.[1,5,6] The transient nature of the biochemical changes makes it difficult to confirm carbamate toxicosis.[6] Therefore, many cases of carbamate poisoning may go unconfirmed. A few cases of carbamate poisoning were reported in livestock, most of which were attributable to carbofuran.[2-4]

In the cows of this report, acetylcholinesterase values failed to support the diagnosis of carbamate toxicosis. Carbamates, in general, are short-acting, reversible inhibitors of acetylcholinesterase. However, little information is available in literature concerning the exact duration of action of aldicarb or carbamates on bovine acetylcholinesterase activity. One cannot extrapolate from data obtained for other species, because a great deal of variation may be observed in acetylcholinesterase response to carbamates. One could speculate that the 4-hour delay between death and analysis could have allowed much of the carbamate-induced inhibition of acetylcholinesterase to reverse with liberation of free acetylcholinesterase. Also, the time between onset of the problem and brain acetylcholinesterase analysis (2 days) could have allowed much of the carbamate-induced inhibition of acetylcholinesterase to reverse.[8]

If carbamate toxicosis is suspected, blood and brain samples should be analyzed immediately for acetylcholinesterase activity. The diagnosis in our cows was hindered by negative results of analysis of rumen contents. Although rumen contents were packed in ice and forwarded for analysis, 4 days of transport time may have been sufficient to allow the breakdown of aldicarb; the laboratory mice actually thrived while ingesting rumen contents. Carbamates, in general, are considered to be rapidly hydrolyzed, reducing the likelihood that parent compounds (aldicarb in this case) would be detected several hours after ingestion. Information is not available regarding the time required for the hydrolysis of these compounds in rumen fluid. Also, aldicarb is such an extremely toxic compound and consequently, death occurred so quickly—often within minutes after ingestion of feed—that this toxic chemical probably would not be thoroughly mixed in the rumen contents. Even though representative samples of rumen contents were obtained, the likelihood still exists that aldicarb was missed by our random sample collection procedure.

The epidemiologic features of interest in this toxicosis are: the condition was confined to lactating dairy cows and developed during milking or within minutes after being milked. Affected cows were of various ages, at various stages of lactation, and had been on the farm for duration ranging from several years to as little as several months. Closer observation during the November and January episodes indicated that affected cows were those that entered the milking parlor first.

The temporal pattern was typical of a common "point" source and was associated with a group of animals being exposed to a common vehicle containing the agent for limited duration. Common-

vehicle epizootics may spread by water, food, air, or inoculation and are characterized by localization in time, place, and animals. Concluding that the common association for all cows was entering the milking parlor, we began a process of ruling out possible vehicles for transmission. Water was not available in the parlor. Food could not be ruled out because cows were fed immediately after entering the parlor, and none had entered with signs of illness. Air was ruled out; none of the personnel and not all cows that came through the parlor at the same time became ill. Problems with the electrical system were not found, and the clinical characteristics of affected cows did not support electrocution. Injections were not given in the parlor on days when cows were affected. Lack of seasonality or known arthropod-borne disease with such rapid onset precluded arthropods as a mode of transmission. We concluded that the causative agent was present in the feed on a sporadic basis. The feed source was a large commercial company, and similar problems had not been observed on other farms in the area. At least 2 drivers delivered feed prior to the episodes. Furthermore, the same load of feed was distributed to several other farms that did not have problems, and death was not related temporally to time of delivery. Specific feeders were involved in the episode; not all cows that entered the parlor first died or became ill. Because various personnel were working in the parlor when the problem was evident, a common shift or employee could not be implicated.

When the owner of the dairy was advised that the only hypothesis that fit the pattern of disease was that of sporadic contamination of specific feeders with a highly toxic substance, he was somewhat suspicious of deliberate poisoning. A high concentration of an antibiotic had been detected in the bulk milk tank during the fall. We were subsequently informed that during the same period, a storage shed was forcefully entered, antifreeze was poured on swine feed, and lines to the milk parlor were broken. Recommendations were made to the owner to secure the feed supply and treat clinically affected animals with atropine. After identifying the causative agent as aldicarb, police began to question employees of the dairy. The person responsible for the introduction of the aldicarb in the feed was subsequently identified.

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