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Case Report – Toxicosis in Cattle Caused by a Discontinued Organophosphorus Insecticide (Fonofos)

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Abstract

Two crossbred cattle were presented to a veterinary teaching hospital in December 2001 with muscarinic, nicotinic and central nervous system signs consistent with severe organophosphorus (OP) insecticide toxicity. Despite treatment, neither animal survived. Over the course of 72 hours, 34 cattle died acutely. A subsequent veterinarian-led visit to the farm identified a rusted, leaking corn planter containing a gray crumbling material, situated near where the cattle were fed. This substance was later identified as fonofos, an agricultural insecticide and an OP that was voluntarily removed from the marketplace by the manufacturer in 1998.

Résumé

Deux bovins de race croisée ont été reçus à l'hôpital vétérinaire d'enseignement en décembre 2001 avec des signes cliniques muscariniques, nicotiniques et du système nerveux central tous compatibles avec un niveau élevé de toxicité relié à un insecticide organophosphoré. Aucun des animaux n'a survécu en dépit du traitement. Dans une période de 72 heures, 34 bovins sont morts de façon aiguë. Une visite subséquente de la ferme menée par un vétérinaire a permis d'identifier un semoir à maïs rouillé et percé contenant un matériau gris se brisant en morceaux près de l'endroit où les bovins étaient nourris. Cette substance s'est révélée plus tard du fonofos, un insecticide organophosphoré utilisé en agriculture mais retiré du marché volontairement par la compagnie manufacturière en 1998.

Introduction

Organophosphorus (OP) compounds became widely used during the 1970s when organochlorine insecticides such as aldrin, chlordane and DDT were phased out in recognition of their damaging environmental impact. The term "organophosphate", which is commonly used for these chemicals, is really a misnomer since they are not salts. Developed originally in the 1930s for use in warfare as nerve gases, OPs are still commonly used as agricultural pesticides for crops as well as systemic parasiticides for livestock. Organophosphorus compounds are toxic because they are cholinesterase inhibitors, binding and inhibiting acetylcholinesterase (AChE), the enzyme that hydrolyzes acetylcholine (ChE), the major cholinergic neurotransmitter. Carbamate pesticides, which are also AChE inhibitors, cause similar clinical signs. However, binding to the enzyme is usually reversible, which is generally not the case with OP insecticides.[4]

Acetylcholine binds muscarinic and nicotinic receptors throughout the central and peripheral nervous systems (CNS and PNS). In the PNS, muscarinic receptors are situated at neuroeffector junctions of the parasympathetic branch of the autonomic nervous system, while nicotinic receptors are found at sympathetic and parasympathetic ganglia and at neuromuscular junctions. Stimulation of a nerve terminal by an action potential causes the release of ChE into the synapse and its binding to one of the two types of receptors. This binding alters influx and efflux of various ions through membrane channels, and enables propagation of the action potential. Normally, AChE effects the immediate breakdown of ChE into its components cho-

line and acetic acid, and halts further stimulation of the nerve.[4]

In the presence of OPs, breakdown of ChE is inhibited by binding of the insecticide to AChE. This causes ChE to accumulate in the nerve synapse, resulting in continuous excitation of receptors and ongoing stimulation of the nerves. Widespread signs of muscarinic, nicotinic and central nervous system toxicity result from these events.

History

A herd of 900 steers and heifers of various ages and breeds were raised on a property in central Indiana. The animals were separated into nine pens, each holding 100 animals of similar age. All cattle were fed a free-choice total mixed ration of silage, 30% corn gluten, and alfalfa hay which the farm manager mixed himself in 2.5-ton batches. Water was provided for each pen in troughs. Animals were previously vaccinated for infectious bovine rhinotracheitis (IBR), parainfluenza 3 (PI3), bovine viral diarrhea (BVD) and bovine respiratory syncytial virus (BRSV) and with a clostridial bacterin-toxoid.

The owner fed a newly mixed ration at noon on the day of the outbreak. Six hours after feeding, he found three animals dead in a pen containing seven-month-old calves and a few older animals. Another hour later, in the same pen, he noticed a two-year old Holstein Friesian heifer shaking, drooling and bellowing, and a seven-month-old beef steer with diarrhea and depression. The owner then brought the three dead animals and the two sick ones to the large animal veterinary hospital at Purdue University.

Upon questioning, the farm owner denied that he had OP insecticides on his property. He had seen what he described as "black specks" in the corn gluten, and was concerned that it was contaminated. He did not bring a sample with him to the hospital.

Clinical Findings

The heifer, whose estimated weight was 880 lb (400 kg), was recumbent and paddling with her front legs. Clinical findings were dyspnea with open mouth breathing, rumen stasis, severe muscle fasciculations, and bilaterally miotic pupils with no pupillary light response or menace. She bellowed continuously, hypersalivated and had a protruding tongue.

The calf, a Limousin steer, estimated weight 440 lb (200 kg), also had severely miotic, non-responsive pupils, hypersalivation and abnormal vocalization. Unlike the heifer whose heart rate was normal, he was tachycardic and had profuse watery diarrhea and a hypermotile rumen. He was neither recumbent nor experiencing muscle fasciculations, but was weak and would occasionally appear ataxic. Breathing was labored, and lung sounds were significantly increased. Both animals had a normal rectal temperature.

Based on history and clinical presentation, the differential diagnoses included ammonia toxicity, carbamate intoxication, grain overload, lead poisoning and organophosphorus insecticide toxicity. Organophosphorus insecticide or carbamate toxicity was considered the most likely. Abnormal lung sounds in the steer suggested underlying and unrelated respiratory disease, such as bacterial or viral pneumonia, lung abscessation or pulmonary fibrosis, but OP-induced increased bronchial secretions was also considered.

Treatment

Both animals were given a poor prognosis. The owner, however, decided to pursue limited treatment. Initial therapy for each animal was 0.5 mg/kg atropine, with 1/4 of the dose administered IV and the rest SQ, an IV injection of flunixin meglumine (1.1 mg/kg) and 1 lb (0.45 kg) of activated charcoal orally. Within minutes of the atropine administration, the steer stopped bellowing and appeared more comfortable, although the majority of clinical signs remained. The heifer did not improve.

A second dose of atropine was given two hours later, and both animals showed clinical improvement. The heifer stopped vocalizing, and retracted her tongue back into her mouth; the steer appeared more alert and drank water. The use of 2-pralidoxime (2-PAM) was discussed with the owner, who declined its use because of cost. He also refused further efforts to diagnose the steer's respiratory disease and rumenotomy to remove any remaining ingested toxin. Rumenotomy for the heifer was not considered because of her poor condition.

Both animals were administered atropine every two hours, and offered fresh water and hay. Unfortunately, the heifer's dyspnea progressively worsened, and she died nine hours after admission.

Because of ongoing diarrhea, normal saline was given IV to the steer at the rate of 1 liter per hour via a jugular catheter. Twelve hours after admission, he appeared significantly improved; heart rate had decreased, manure was firmer and he ate a small amount of hay. Another pound of activated charcoal was administered along with a second dose of flunixin meglumine. Fluids and atropine were discontinued.

Forty-eight hours after admission, the steer became dyspneic. Because of uncertainty about the cause of the dyspnea, atropine therapy was resumed. The steer was also given 10 mg of dexamethasone IM and 250 mg of furosemide for suspected pulmonary edema. Despite treatment, the animal died six hours later.

Both animals were sent for necropsy to the Purdue University Animal Disease Diagnostic Laboratory. Blood cholinesterase activity by UV spectrophotometry, drawn immediately post-mortem, was 2% of normal in the heifer and 1.8% of normal in the steer. Brain cholinesterase in the heifer was 14% of normal. Brain cholinesterase activity in the dead calves submitted directly for necropsy was similarly low. Analysis of rumen contents confirmed the presence of a large amount of fonofos. Both the heifer and steer had pulmonary edema. The steer also had pulmonary abscesses. Viral and bacterial cultures from these abscesses were negative.

A subsequent visit to the farm resulted in the discovery of a gray crumbling substance in a large metal corn planter near the pen that had held the affected animals. The bottom of the planter had rusted through, and the contents had spilled into the area where the affected animals were fed. Toxicological analysis identified this compound as fonofos. The hay had been placed atop the fonofos, and the animals had ingested the chemical as they consumed the ration. The owner had no recollection of either the purchase of the insecticide or its storage in the planter. In total, 34 cattle from one pen containing 100 animals died acutely from fonofos ingestion. The feed itself did not contain any fonofos, and no evidence of the black specks in the feed reported by the owner was found.

Discussion

Organophosphorus compounds have been the cause of numerous livestock deaths since they were introduced in the 1930s. Between 1981 and 1991, several reports of fonofos poisoning of cattle were published, most of which resulted from accidental feed contamination due to improper labeling, storage or disposal of the chemical.[2,7,8,10,18,19]

This report highlights the fact that stocks of discontinued OPs still exist, and are still potent. Fonofos, chemical name O-ethyl S-phenyl ethylphosphonodithioate, was introduced in the 1970s as an insecticide against rootworms, cutworms and other soil-dwelling pests. Applied directly to the ground, and used primarily for corn and other vegetable crops, it was sold in a variety of formulations and under several different trade names.[3,7,a] Fonofos was listed as a restricted use chemical by the EPA, as well as a Class I Toxin, and was determined to be extremely hazardous to birds, aquatic organisms and bees with an oral LD50 in dairy cattle of 1.30mg/kg. There have also been several reports of accidental poisonings in humans. It has a long half-life in soil of up to 435 days. In 1998, the chemical was voluntarily withdrawn from the marketplace prior to its reregistration by its manufacturer The EPA allotted the company one year to dispose of its stores and allowed individuals who possessed stocks of the chemical to continue its use until gone.[3]

The mneumonic DUMBELS, (diarrhea/dyspnea, urination, miosis, bradycardia, emesis, lacrimation and salivation) is sometimes used to describe the PNS muscarinic signs of OP toxicity, while the MTWHF mneumonic (mydriasis, tachycardia, weakness, hypertension, fasciculations) is employed for the characteristic PNS nicotinic signs.[12] Clearly, several of these signs are contradictory, and animals with OP poisoning may show a combination of muscarinic and nicotinic signs concurrently. While seizures are extremely rare in food animals, signs such as hyperreflexia, abnormal vocalization and atypical behavior, such as severe aggression, can also be present, representing what are termed CNS signs.

Severity of the clinical signs is attributable to dosage, to the specific OP involved, to the age of the animal – younger animals usually are more seriously affected— and to the mode of exposure. Organophosphorus compounds can be absorbed through skin, orally or via inhalation. Accidental ingestion of the chemical is most common in livestock, which is usually the most toxic route of exposure.[9] In general, the faster the signs of poisoning appear, the worse the intoxication.[12] Signs can appear within 30 minutes of exposure, usually by six hours, and almost certainly within 12 hours.[1] Severe cases result in respiratory failure, which can be centrally mediated, due to nicotinic paralysis of respiratory muscles, or the consequence of muscarinic excess bronchial secretions.[5]

Affected animals in other oral fonofos intoxication cases demonstrated similar combinations of muscarinic, nicotinic and CNS signs as described here. Clinical signs displayed by the heifer were a combination of muscarinic signs (miosis, salivation), nicotinic signs (muscle fasciculations) and CNS signs (paddling, recumbency). The steer had additional muscarinic signs (diarrhea, hypermotile rumen), nicotinic signs (tachycardia) and CNS signs (weakness, ataxia). The dyspnea could have either been muscarinic and due to increased bronchial secretions, of CNS origin, or both. The rumen stasis displayed by the heifer was probably not due specifically to OP toxicity, but because the animal was so ill. Other reported signs of fonofos poisoning in food animals include nasal discharge, coughing, vomiting, bradycardia, lacrimation, hematochezia, tenesmus, polyuria and opisthotonus, none of which were seen in the animals in this report. It is common for a variety of clinical signs to be seen within a group of animals. This can be due to the age of the animal or the quantity ingested, and possibly to other factors such as gender, diet and general health.[9,18]

The initial improvement of the steer following atropine therapy suggests that rumenotomy may have

been helpful. However, pulmonary abscessation identified at necropsy indicated that he was already significantly debilitated, and that recovery might well have been compromised regardless of therapy. Many intoxicated animals have no significant gross lesions at necropsy, although pulmonary edema is a common finding in OP poisoned animals, along with the presence of the toxin within the digestive tract.[8]

Diagnosis of OP ingestion is done by chemical analysis of rumen content and feed, and by measurement of cholinesterase activity in brain tissue. In addition, because red blood cells contain acetylcholinesterase, measurement of blood AChE is a useful antemortem test.[22] An 80% reduction in normal blood cholinesterase activity (normal bovine measurements range from 3.968 ± 0.8999 uM/g/min by the Ellman method) is strongly suggestive of acute OP intoxication, but is not quantitative since the degree of erythrocyte AChE inhibition does not correlate with the amount of OPs absorbed.[7] Plasma is not recommended for testing, since it contains a different type of cholinesterase, called a pseudo-cholinesterase, which exhibits changes in activity that are not necessarily correlated to toxicity. Refrigerated whole blood samples can be evaluated up to one week after collection.

Post-mortem OP toxicity can be best diagnosed from measurement of ChE activity in brain samples. Whole brain or one hemisphere should be submitted because ChE activity is not evenly distributed throughout the brain. A whole eye can be helpful for diagnosis since the retina can be tested for AChe activity. Samples should be refrigerated and not frozen. Rumen and feed samples should be frozen after collection and submitted in glass or metal containers, since OPs can leach from plastic.[15]

Both atropine sulfate and oximes — most commonly, 2-pralidoxime (2-PAM) — are antidotes commonly used to treat OP poisoning in domesticated animals.[20,21,22] Although they can be used independently, the two drugs are synergistic and are commonly used together.

Atropine, a muscarinic receptor antagonist, blocks the muscarinic signs and some of the CNS signs, but not the nicotinic effects of OP toxicity. Generally, the total dose of atropine (0.1 to 0.5 mg/kg) is split so that one-fourth to one-third of the total amount is given IV and the rest IM or SQ. This quantity of atropine, however, can itself cause signs of toxicity including rumen stasis, respiratory depression and tachycardia.[14] As a result, the clinician can be faced with the conundrum of determining whether the initial clinical presentation could be worsened by the treatment, and further, whether abnormalities seen after treatment were caused by the original intoxication or by the atropine. Atropine was administered to the heifer with the awareness that it could potentiate the rumen stasis, but with the hope that it would alleviate some of the more immediately life-threatening problems. Since both animals also had significant nicotinic and CNS signs of OP intoxication, the limited improvement seen following atropine administration was not unexpected.

2-pralidoxime functions by breaking the OP-AChE phosphoryl bond and reactivating AChE. Thus, it can alleviate muscarinic, nicotinic and CNS signs of toxicity. The success of 2-PAM at the recommended dose of 13.6 mg/lb (30 mg/kg) depends upon the severity of the poisoning and the time between poisoning and initiation of treatment. OP-AChE complexes become more strongly bound with time, a phenomenon known as "aging." After this has occurred, 2-PAM cannot release AChE from its OP bound state. Twenty-four hours is usually considered the limit for successful use of 2-PAM.[15]

2-pralidoxime is also rarely effective in animals with severe toxicity, which may be due to its inability to cross the blood-brain barrier.[22] While 2-PAM might have helped these animals initially, it likely would not have been curative because of the large quantity of OP remaining in the rumen and the severity of the intoxication. Unfortunately, the high cost of 2-PAM often makes its use in food animals prohibitive, as was the case here.

Additional treatment for OP intoxication in cattle includes oral administration of activated charcoal at a dose of 1 to 2 lb (0.45 to 0.91 kg) per adult animal, washing with soap if dermal exposure if suspected and supportive care.[4,15]

Carcass disposal in lethal OP poisonings can be problematic because the animals cannot be rendered for feed. OPs do not dissipate readily in dead animals and incineration or burial may be required, along with additional toxicological tests for residues.[17] Veterinarians and producers faced with these situations need to contact their state veterinarian's office, as well as FARAD, to obtain specific recommendations. The fate of survivors of OP intoxication can be equally confounding. Since most OP compounds do not accumulate in meat or milk, surviving animals may be safe for slaughter or milk production within a few days of recovery.[8] With almost all OP pesticides, including fonofos, highest residue levels occur in the liver and kidney.[7] Studies have shown that laboratory animals fed small but still toxic amounts of fonofos, will excrete almost all of the chemical in urine and feces within 96 hours. However, a few OP pesticides are lipophilic and residues can remain in the body for days to weeks. Furthermore, there is considerable evidence that rodents and food animals have significantly different sensitivities to OPs (including fonofos) which suggests that results obtained from one species cannot necessarily be applied to another.[6] Here, too, contacting both the state veterinarian and the di-

agnostic laboratory is a necessity following a confirmed OP intoxication to determine specific regulations for surviving livestock. Decisions as to whether to destroy survivors, to utilize mandatory withdrawal times, or to test tissues are usually performed on a case-by-case basis.

Similarly, practitioners treating food animal toxicities must consider withdrawal times for antidotes administered. The Food Animal Residue Avoidance Databank (FARAD) has reviewed data relevant to withdrawal times for antidotes and is available for consultation (1-888-873-2723 or farad@ncsu.edu). Based on published studies in pigs, sheep and mice, FARAD recommends the United Kingdom's 6-day milk and 28-day meat withdrawal times for typical atropine use as an antidote. FARAD has also reviewed available pharmacokinetic data for 2-PAM when it is used to supplement atropine therapy in OP intoxications and finds that the withdrawal times recommended for atropine are appropriate for 2-PAM as well (Michael Payne, DVM, PhD, FARAD, Pers. Comm.).

Footnote

[a] Tradenames for fonofos include Tycap, Cudgel, Capfos, Difonate, Dyfonate, Dyphonate and Stauffer N 2790. Its original manufacturer was Stauffer Chemical Company (1967). Later, manufacturing was taken over by Zeneca Ag Products.

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