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Brodifacoum toxicosis and abortion in an Arabian mare

Amir Zakian[1]*, Sajad Mami[2], Mohammad Nouri[3], Seyedeh Misagh Jalali[3], Meysam Tehrani-Sharif[4]

[1] Department of Clinical Sciences, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran; [2] Department of Laboratory and Clinical Sciences, Faculty of Veterinary Medicine, Ilam University, Ilam, Iran; [3] Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran; [4] Department of Clinical Sciences, Faculty of Veterinary Medicine, Islamic Azad University, Garmsar Branch, Garmsar, Iran.

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Abstract

A 3-year-old pregnant Arabian mare was referred to the Veterinary Teaching Hospital of Shahid Chamran University of Ahvaz with a history of bleeding and rodenticide ingestion. The results of paraclinical examinations showed severe normocytic and normochromic anemia, decreased serum total protein, albumin, and fibrinogen concentrations, increased serum total bilirubin, urea, and creatinine concentrations, as well as increased serum aspartate aminotransferase and creatine kinase activity. Three days after treatment, all the clinical signs were resolved, however, fetus abortion occurred. In order to confirm the suspected cause of abortion and toxicosis, high-performance liquid chromatography was performed on serum sample of mare and liver tissue of the aborted fetus and toxicosis was confirmed. Poisoning with brodifacoum is considered as an important and lethal poisoning for both human being and animals. To our knowledge, this is the first report of spontaneous toxicosis and abortion with brodifacoum. Brodifacoum toxicosis can be effectively managed with early diagnosis, good paraclinical examinations and appropriate treatment.

مسمومیت با برادیفاکوم و سقط در یک رأس مادیان عرب

چکیده

مادیان عرب سه ساله ای با تاریخچه خونریزی و مصرف جونده کش به بیمارستان آموزشی دامپزشکی دانشگاه شهید چمران اهواز ارجاع داده شد. نتایج آزمایشات پاراکلینیکی تغییرات برخی فراسنجه های هماتوبیوشیمیایی از قبیل کم خونی شدید نورموسیتیک و نورموکرومیک، کاهش غلظت سرمی پروتئین تام و فیبرینوژن، افزایش غلظت سرمی بیلی روبین تام، اوره و کراتینین و همچنین افزایش فعالیت آنزیم‌های آسپارتات آمینوترانسفراز و کراتین کیناز را نشان داد. سه روز پس از شروع درمان، تمامی علایم بالینی برطرف شد اما متاسفانه سقط جنین رخ داد. به منظور تایید علت احتمالی سقط به روش کروماتوگرافی با عملکرد مایع بالا نمونه سرم مادیان و نمونه بافت کبدی جنین سقط شده بررسی شد و مسمومیت تایید گردید. مسمومیت با برادیفاکوم به عنوان یکی از مسمومیت‌های مهم و کشنده در حیوانات و انسان مورد توجه می باشد. براساس اطلاعات ما، گزارش حاضر اولین مورد مسمومیت خودبخودی توام با سقط جنین ناشی از جونده کش برادیفاکوم است. با بهره بردن از آزمون های پاراکلینیکی مناسب در مراحل ابتدایی می‌توان مسمومیت با برادیفاکوم را تشخیص داد و در صورت درمان صحیح، به راحتی قابل مدیریت است.

واژه های کلیدی: اسب عرب، برادیفاکوم، جونده کش، کروماتوگرافی مایع با عملکرد بالا، هماتوبیوشیمی

***Correspondence:**

Amir Zakian. DVM, DVSC
Department of Clinical Sciences, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran
E-mail: zakian.a@lu.ac.ir

Introduction

Anticoagulant rodenticides can be categorized based on their chemical structures into two main groups: Hydroxycoumarine and indandinone (chlorophacinone, diphacinone, pindone, and valone). Hydroxycoumarin is divided into first-generation anticoagulant rodenticides (FGAR) such as coumachlor, coumafuryl, coumatetralyl, and warfarin and second-generation anticoagulant rodenticides compounds (SGAR) like brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen.[1,2] Brodifacoum has the lowest reported oral median lethal dose (LD_{50}) among all the anticoagulant rodenticides.[1,2] Its LD_{50} is estimated to be 50 to 100 mg per adult horse[3] due to good absorption from the gastrointestinal tract.[4] The time to the peak level of plasma concentration for this toxin is within 12 hr of ingestion. After absorption, it is rapidly distributed and could be detected in a stable form in different tissues such as liver and kidney. Its excretion rate is very slow.[5]

Few reports of anticoagulant intoxications in horses are available in peer-reviewed publications,[1,2,6,7] and also we could not find any reported case of brodifacoum toxicosis which led to abortion in the horse. In the present report, we described a clinical case of accidental ingestion of brodifacoum substance in an Arabian horse that resulted in abortion.

Case Description

History. A 3-year-old Arabian mare, weighting about 300 kg, carrying a 7-month-old fetus, was referred to the Veterinary Teaching Hospital of Shahid Chamran University of Ahvaz, Ahvaz, Iran. The animal had a history of poor appetite, depression, and hemorrhage from the mouth, nostrils, and left hind limb. According to the owner statments, the total quantity of diet was 8-9 kg of good quality alfalfa hay and 4 kg of cereal-based concentrate which was divided into three meals per day. The mare had a body condition score of five based on the modified 9-point Henneke scale.[8]

Clinical findings. The clinical symptoms observed during the examination were pawing, stretching, colic, mild dehydration, oliguria, increasing concentration of urine, constipation, congestion of mucous membranes with petechia of the gums, restlessness, nystagmus, trismus, and tremor associated with the short periods of excitation. The mare had a moderate tachycardia (60 beats per min), mild hypothermia (rectal temperature was 37.10 ˚C) and capillary refill time was 3 sec. Auscultation and percussion of abdomen indicated distention with gas and ileus of right side paralumbar fosa region. Passage of a nasogastric tube did not yield reflux of gas or stomach contents. Lungs sounds were normal. Stiff gait and lameness were observed with no sign of paresis, however, slight hypermetria in all four limbs was noted and hemarthrosis in left forelimb was seen. In the rectal examination, no abnormality was detected and the fetal position was normal. A blood sample was collected from the left jugular vein using 10 mL disposable syringe and 18-gauge needle. Serum and plasma samples harvested after centrifugation. Prolonged bleeding (10 min) from the puncture site of blood collection occurred which was finally controlled by the application of manual pressure.

According to the owner, 54 hr before presentation the rodenticide, Facorat pellets (I.N.D.I.A. Industrie Chimiche Co., Veneto, Italy), containing 0.005% brodifacoum, was used for control of rodents on the farm. Based on the clinical signs and the history provided by the owner, we hypothesized that the rodenticide might have been accidentally mixed with feed and ingested by the horse.

Diagnostic procedures. Hematology, coagulation tests, clinical chemistry, urine, and peritoneal fluid analyses were performed together with blood parasite test and fecal examination (Table 1). The hematology analyses showed a severe normocytic, normochromic anemia. Blood coagulation profile revealed prolongation of prothrombin time (PT) and activated partial thromboplastin time (aPTT), indicative of impairment of both intrinsic and extrinsic coagulation pathways. No parasitic infection was detected on the blood smear.

Decreased serum total protein, albumin, and fibrinogen concentrations and increased serum total bilirubin, urea, lactate and creatinine concentration, aspartate aminotransferase (AST) and creatine kinase (CK) activity, were observed in serum biochemical analysis.

Urinalysis was performed using a rapid urine test strip (Medi-Test Combi 10® VET; Macherey-Nagel GmbH & Co. KG, Duren, Germany). The results of urinalysis revealed hematuria (more than 10 and less than 50 RBC per μL), proteinuria (trace, less than 30 mg dL^{-1}) and glucosuria (trace, 50 mg dL^{-1}).

Peritoneal fluid was collected through abdominocentesis using a 10 cm catheter with a gauge of 16. Peritoneal fluid was reddish in appearance (Fig. 1) with a high protein concentration and high RBC count. No evidence of gastrointestinal parasitism was detected with McMasters technique[9] in the fresh feces. Careful diagnostic aspiration of left forelimb knee with 20-gauge needle showed hemarthrosis.

Using high-performance liquid chromatography (HPLC) and difenacoum as the internal standard at flow rate of 1 mL min^{-1} in 45 ˚C with 0.2 M acetonitrile-N-propanol, ammonium acetate as mobile phase and pH 4 buffer (62:3:35); using fluorescence detector under 40 bar pressure, high level of brodifacoum was found in the plasma sample of the mare and the liver tissue of the aborted fetus (6.80 μg L^{-1} and 2.10 ng g^{-1} of brodifacoum, respectively).

Fig. 1. The bloody peritoneal fluid of poisoned mare with history of bleeding and rodenticide ingestion.

Treatment and outcome. Based on the history, clinical signs and laboratory results presumptive diagnosis of rodenticide toxicosis was established. The mare was treated with intravenous flunixin meglumine (0.50 mg kg^{-1}, q24hr; Rooyan Darou Pharmaceutical Co., Tehran, Iran), subcutaneous vitamin K1 (2.50 mg kg^{-1}, q24hr; Caspian Tamin Co., Tehran, Iran), single dose of intramuscular cyanoferin (15.00 mg kg^{-1}; Nasr Pharma Co., Fariman, Iran), vitamin B complex (4.00 mg kg^{-1}, q24hr; Rooyan Darou Pharmaceutical Co., Tehran, Iran) plus oral administration of 3.00 L of mineral oil (Rooyan Darou Pharmaceutical Co., Tehran, Iran) and psyllium mucsiloid (500 mg kg^{-1}; Dineh pharmaceutical Co., Tehran, Iran) by nasogastric tube. Pharmacotherapy was followed by fluid therapy with isotonic polyionic solutions 4.00 L for three consecutive days. Treatment with subcutaneous vitamin K (1.00 mg kg^{-1}, q24hr) and intramuscular B complex (0.20 mL kg^{-1}, q48hr) was continued until the disappearance of clinical symptoms. Three days after the onset of clinical signs, abortion occurred. Necropsy and diagnostic investigation of the aborted fetus and placenta did not confirm any findings on infectious disease. Ten days after treatment, all hematological, biochemical and coagulation parameters were within the reference ranges (Table 1) and no sign of bleeding was noted, however, treatment with vitamin K continued for three weeks.

Table 1. Results of hematology, coagulation, clinical chemistry, and peritoneal fluid analyses of a mare with brodifacoum toxicosis before and 10 days after treatment.

Analytes	Before	After	RI[6,7,10]
Hematology			
RBC (× 10^{12} L^{-1})	4.19	6.78	6.80 - 12.90
HGB (g L^{-1})	59.10	127.00	110.00 - 190.00
HCT (%)	18.00	49.00	32.00 - 53.00
MCV (fL)	42.90	50.90	37.00 - 59.00
MCH (Pg)	14.00	16.00	12.30 - 19.70
MCHC (g L^{-1})	32.90	33.70	31.00 - 38.60
RDW (%)	18.00	18.00	17.00 - 20.00
PLT (× 10^{3} μL^{-1})	121.00	291.00	100.00 - 600.00
MPV (fL)	5.90	6.90	5.40 - 9.30
PDW (%)	39.00	47.00	24.00 - 72.00
PCT (%)	0.08	0.12	0.07 - 0.21
WBC (× 10^{9} L^{-1})	8.60	6.50	5.40 - 14.20
Neutrophils (× 10^{9} L^{-1})	3.52	4.78	2.30 - 8.50
Lymphocytes (× 10^{9} L^{-1})	5.07	6.19	1.50 - 77.00
Coagulation			
PT (Sec)	>30.00	<10.00	8.50 - 9.90
aPTT (Sec)	>65.00	<40.00	30.00 - 44.00
Biochemistry			
Fibrinogen (g L^{-1})	1.00	3.00	2.00 - 4.00
Total Protein (g L^{-1})	52.00	61.00	60.00 - 77.00
Albumin (g L^{-1})	26.00	33.00	29.00 - 38.00
Urea (mmol L^{-1})	4.83	3.60	3.50 - 7.00
Creatinine (mmol L^{-1})	179.46	141	110.00 - 170.00
Total Bil (mmol L^{-1})	38.82	28.40	7.10 - 35.00
Direct Bil (mmol L^{-1})	3.25	1.25	0.00 - 6.80
Calcium (mmol L^{-1})	2.85	3.25	2.80 - 3.44
Phosphorous (mmol L^{-1})	1.13	0.94	0.7 - 1.68
Magnesium (mmol L^{-1})	0.98	1.05	0.74 - 1.20
Glucose (mmol L^{-1})	4.60	4.89	4.20 -6.40
Lactate (mmol L^{-1})	1.92	1.73	1.10 - 1.80
CK (U L^{-1})	323	315	145 - 380
ALP (U L^{-1})	1527	1500	140 - 4003
AST (U L^{-1})	698	521	220 - 600
ALT (U L^{-1})	10	8.00	3.00 - 23.00
Peritoneal fluid			
Color	Reddish	Clear/Yellowish	Clear/Colorless
Protein (g L^{-1})	30.00	17.00	5.00 – 25.00
WBC (× 10^{9} L^{-1})	< 0.005	< 0.005	0.005 - 0.05
RBC (× 10^{12} L^{-1})	>1	0	0

RBC: red blood cell; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; PLT: platelet count; MPV: mean platelet volume; PDW: platelet distribution width; PCT: platelet-crit; WBC: white blood cell; PT: prothrombin time; aPTT: activated partial thromboplastin time; Bil: bilirubin; CK: creatine kinase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; RI: reference interval.

Discussion

The most common causes of toxin-induced hemorrhagic syndrome in horses are poisoning with warfarin or other rodenticides and vitamin K deficiency. Clinical signs of the hemorrhagic syndrome may include: bleeding, congested and/or pale mucous membrane, abortion, and recumbency.[10] Clinical signs generally would not develop until 1-2 days or five days after ingestion until depletion of the active clotting factors.[7,11] In the current case, clinical signs in the poisoned mare appeared in approximately 54 hr after ingestion of anticoagulant.

Brodifacoum exerts its effects by interfering with reactivation of vitamin K.[1,3] that is necessary for the production of clotting factors such as II, VII, IX,[1-3,7] by which proteins C and S will be competitively inhibited. Thus, prolongation of blood clotting time occurs and

spontaneous hemorrhage of tissues that contain vitamin K-epoxide reductase (e.g. liver, kidney, and pancreas) is seen.[4,6] In the present case, prolonged PT and aPTT returned back to normal reference ranges seven days after treatment. It has been reported that concentrations of PT and aPTT were significantly prolonged between days 4 to 8 after intoxication,[6] however, in the current case, prolongation of PT and aPTT occurred within three days after intoxication.

Changes in body temperature (BT) in brodifacoum poisoning has been a contradictory finding and a decreasement[2] or increasement[3] in BT have been reported. In this case, hypothermia was present. The most likely causes of the abortion in this case, should have been severe hypoxia from the mare's anemia, fetal bleeding in the fetus and placenta, hypothermia or effect of intoxication on the fetal liver.

Decreased red blood cell parameters in our case indicated severe anemia likely due to hemorrhage as reported previously.[6] Decreased level of total protein, albumin, and platelet content in this mare indicated an active and ongoing hemorrhage. Mild increases in AST and CK activities and total bilirubin concentration in the present case could be indicative of liver injury as reported previously.[7] The marked increase in indirect bilirubin could be likely from RBC breakdown from internal hemorrhage.

The definitive antidote of brodifacoum is vitamin K1. However, administration of vitamin K3 (menadione) was useful and had side effects in cases with water deficiency because menadione is highly nephrotoxic in horses[12-14] and unable to serve as a cofactor for the hepatic vitamin K-dependent carboxylase. Thus, vitamin K3 is contraindicated for the treatment of rodenticides. Treatment with vitamin K should be continued until coagulation status is normal, 2 - 3 days after the last dose of K1.[12]

In rodenticide toxicosis, anti-inflammatory drugs (NSAID) should be administrated with caution and in low doses, because some factors like highly protein-bound or disease states such as hypoproteinemia or chronic renal disease may increase susceptibility to poisoning.[7,13,14] After two days, the prognosis of SGAR was more favorable, and the recovery of the clotting factors was a sign that the animal had overcome the intoxication by anticoagulant rodenticides.[14]

To the best knowledge of the authors, this was the first report of brodifacoum toxicosis in the mare and the fetus which resulted in abortion. The present case also showed that with early diagnosis and appropriate treatment, toxicosis could be successfully managed.

Conflict of interest

None of the authors has any conflict of interest in the present study.

References

1. Valchev I, Binev R, Yordanova V, et al. Anticoagulant rodenticide intoxication in animals: a review. Turk J Vet Anim Sci 2008; 32(4): 237-243.
2. Carvallo FR, Poppenga R, Kinde H, et al. Cluster of cases of massive hemorrhage associated with anticoagulant detection in race horses. J Vet Diag Invest 2015; 27(1): 112-116.
3. Murphy MJ, Gerken DF. The anticoagulant rodenticides. In: Kirk RW (Ed). Current veterinary therapy X: Small animal practice. Philadelphia, USA: WB Saunders 1989;143-146.
4. Stone WB, Okoniewski JC, Stedelin JR. Poisoning of wildlife with anticoagulant rodenticides in New York. J Wildl Dis 1999; 35(2): 187-193.
5. Maroni M, Colosio C, Ferioli A, et al. Biological monitoring of pesticide exposure: a review. Toxicology 2000; 143(1): 1-118.
6. Boermans HJ, Johnstone I, Black WD, et al. Clinical signs, laboratory changes, and toxicokinetics of brodifacoum in the horse. Can J Vet Res 1991; 55(1): 21-27.
7. Ayala I, Rodriguez MJ, Martos N, et al. Fatal brodifacoum poisoning in a pony. Can Vet J 2007; 48(6): 627-62.
8. Henneke DR, Potter GD, Kreider JL, et al. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet J 1983; 15(4): 371-372.
9. Zajac AZ, Conboy GA. Veterinary clinical parasitology. 8th ed. Philadelphia, USA: Wiley-Blackwell 2012: 8-11.
10. Constable PD, Kenneth W, Hinchcliff K, et al. A textbook of the disease of cattle, horses, sheep, pigs and goats. 10th ed. Madrid, Spain: WB Saunders 2017: 1839-1840.
11. Binev R, Petkov P, Rusenov A. Intoxication with anticoagulant rodenticide bromadiolone in a dog – a case report. Vet Arch 2005; 75(3): 273-282.
12. Hanslik T, Prinseau J. The use of vitamin K in patients on anticoagulant therapy: a practical guide. Ame J Cardiovas Drugs 2004; 4: 43-55.
13. Rebhun WC, Tennant BC, Dill SG, et al. Vitamin K3-induced renal toxicosis in the horse. J Am Vet Med Assoc 1984; 184(10): 1237-1239.
14. McConnico RS, Copedge K, Bischoff KL. Brodifacoum toxicosis in two horses. J Am Vet Med Assoc 1997; 211(7): 882-886.