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CASE REPORT

A CASE OF INTOXICATION WITH THE ANTICOAGULANT RODENTICIDE BROMADIOLONE IN A DOG

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Pesticides are used in different branches of agriculture, industry, health care etc. Their wide application and the inadequate information about the means of their use and their toxic effects are a real risk for the health of both animals and humans (Maroni *et al.*, 2000).

From the point of view of ecological agriculture, the control of harmful rodents (mice, rats, field mice etc.) requires a precise and adequate use of chemical means of restriction of their population. By now, the use of baits containing various active substances with anticoagulant effect is the most frequent (Sengalevich *et al.*, 1998).

Recently, anticoagulant rodenticides, causing severe injury of vascular permeability resulting in massive haemorrhages and rapid death of rodents (Nieuwenhuys *et al.*, 2000; Zhou and Chan, 2001) are most widely used.

Until now, cases of intoxication with anticoagulant rodenticides and their effects except in mice and rats (Nieuwenhuys *et al.*, 2000; Howe and Webster, 2000) were described also in hamsters (Shan and Suttie, 1975), cats (Smith *et al.*, 2000a, Smith *et al.*, 2000b), human volunteers (Lehman, 2000), in wild animals (deers, coons, martens, eagles, owlets, hawks etc.) with various anticoagulants as bromadifacoum, chlorophacinone, bromadiolone and coumatetralyl (Stone *et al.*, 1999).

A spontaneous intoxication with an unknown anticoagulant rodenticide in a dog have been described by Blocker and Roberts (1999) characterized with massive haemorrhages in the trachea and pulmonary parenchyma, resulting in obstructions.

One of most commonly used rodenticides is bromadiolone, included in the composition of the commercial preparation Lanirat (Ciba-Geigy, Swiss). It is used (2 L Lanirat 0.005) added to a bait consiting of 100 kg ground grain, 1.5 L sunflower oil and 4 L water (Sengalevich *et al.*, 1998) or to other kinds of baits.

The literature data concerning complete clinical and laboratory studies on the spontaneous intoxication with bromadiolone in dogs are insufficient. This motivated our complex clinical, laboratory, microbiological, radiological and ultrasonographic investigations in order to facilitate the diagnostics and the treatment of dicoumarin anticoagulant intoxications.

On March 11, 2002 a 18-month-old male Caucasian shepherd dog originating from the town of Shoumen, named Spike, weighing 60 kg, was referred to the Clinic of Internal Diseases at the Faculty of Veterinary Medicine, Trakia University - Stara Zagora. The anamnesis revealed that a week ago, the owner had noted a symmetrical abdominal enlargement, decreased appetite, frequent urination and

increased thirst. Another 3-4 weeks ago, a deratization by placement of baits containing Lanirat 0.005 in farm premises, where the dog was used as a guard, had been performed. Later, the dog was occasionally noticed eating dead and dying rodents. It was fed with granulated food and plenty of meat and fats. No ill dogs were present in the neibourhood. The dog has not been ill prior to the incident. The immunoprophylactic and antihelminthic drugs were regularly administered. During the period prior to the referral, the body temperature was increased up to 40 ^{o}C. The dog was given single doses of antibitoics - ampicillin (Shotapen) i.m. and Penicillin 2 000 000 UI intraperitoneally, but with no effect.

During the hospitalization, the clinical status was followed twice daily by respect to body temperature (BT), heart rate (HR) and respiratory rate (RR), the colour of mucosae, appetite, thrist, general condition, locomotor activity, sensory perception, urination, defecation etc. using routine clinical diagnostic methods.

At referral and at post hospitalization days 4, 8, 12 and 16, blood from *v. cephalica antebrachii* was sampled for determination of haemoglobin concentration (Hgb), red blood cell (RBC) counts, white blood cell (WBC) counts and haematocrit values (Hct) (using an automated haemoanalyzer Serono-System 150+, USA), the differential WBC counts (by the method of Pappenheim), erythrocyte sedimentation rate (ESR) (by the method of Panchenko), thrombin time (with diagnostic kits of Hemostat TT, Human, Germany and a KC 1 A coagulometer, Amelung). The activities of transaminases ASAT and ALAT, total bilirubun, urea, creatinine, blood sugar, were assessed on an automated analyser Reflotron Manual (Germany) using diagnostic kits of Roche (Germany). A functional analysis of liver was performed by the following liver function tests - Lugol's test (Mallen and Wool), formaldehyde test (Gate and Papacostas), cadmium test (Wunderly and Wuhrmann) and acetone test (Nikov), described by Anguelov *et al.* (1999).

The course of the disease and the effect of the treatment were further followed out by thoracic and abdominal radiography (TUR D-800-1, Germany) and ultrasonography (Aloka-SSD-500, UST-5871-5 probe).

Several punctions of the thorax (in the left intercostal spaces VII–VIII and the right intercostal spaces IV–V) and the abdomen were performed at hospitalization and post hospitalization days 3, 6, 9 and 12. The physical analysis of punctate included its colour, transparency, density (by an aerometer) while the chemical analysis – the amount of protein (Esbach's albuminometer). The punctate was microscopically and microbiologically studied on McConkey agar (blood agar with 5% sheep red blood cells). The Rivolta's test was applied for differentiating transudate from exudate.

The physical examination revealed pale conjunctives, decreased locomotor activity and sensory perception, rapid exhaustion, bilateral symmetrical abdominal enlargement with drooping of the ventral wall and spinal lordosis. The patient was constantly sitting in order to ease the respiration. The body temperature was 39.6 ^{o}C, the heart rate – 115 min^{-1} and the respiratory rate - 88 min^{-1}

During the hospitalization (Fig. 1) an elevated BT during the first two days was present, but afterwards, until the end of the follow-up, it was within the physiological range (37.5–39.0 ^{o}C).

A tachycardia was observed from the first (115 min^{-1}) until the 7th (82 min^{-1})

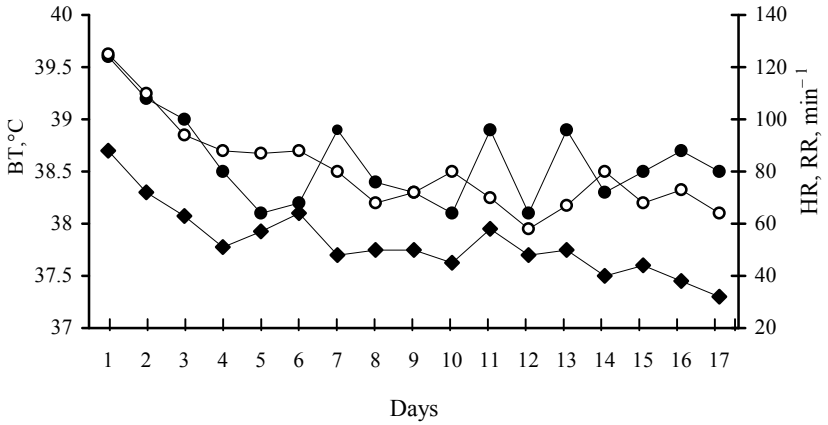

Fig. 1. Changes in body temperature (–♦–), heart rate (–o–) and respiratory rate (–●–) of a dog following intoxication with the anticoagulant rodenticide bromadiolone.

day of the hospitalization. From day 8 until the end of the follow-up, the RR was normalized (60–80 min^{-1}). The pulse was strong, full, hard and rhythmic. After percussion, a decrease in the absolute cardiac dullness was observed while the auscultation revealed stunt cardiac tones.

The breathing at referral was with increased frequency (88 min^{-1}), difficult (with open mouth) from a costo-abdominal type with the active participation of abdominal muscles in both respiratory phases. The percussion showed a dullness in the ventral pulmonary areas with a horizontal upper limit that was moving with lifting of the front part of the body. The auscultation evienced a bronchial respiration with moist fine bubbling rales over the limit of the horizontal dullness. Underneath, the respiratory sounds were poorly audible. The RR was enhanced (polypnea) for the whole period of observation (Fig. 1). The tendency towards lower RR observed during the second half of the period did not result in normalization and the values were still over the physiological limits (15–25 min^{-1}).

The thrombin time was 18.2 min at the beginning of the period of observation. Following the treatment, it restored within the reference range (2–6 min) by day 13 of the hospitalization.

Lateral thoracic and abdominal radiographs was performed at referral, that showed a shading with horizontal upper limit.

An ultrasonography was also performed, to specify the amount and the position of the liquid in the thoracic and abdominal cavities (Fig. 2). The interpretation suggested vast anechogenic spaces (filled with liquid), contrasting to the more echogenic zones of the liver, spleen, intestines and the soft abdominal wall. Similar echographic picture was observed in the pleural cavity as well.

At day 12, a second ultrasonography was performed (Fig. 3), that evidenced a marked decrease of thoracic and abdominal liquids.

The optimal site of thoracic punction was found out using general and special methods. It was located in the left VIIIth intercostal space in the zone of maximum dullness. During the hospitalization, 5 thoracic punctions have been performed –

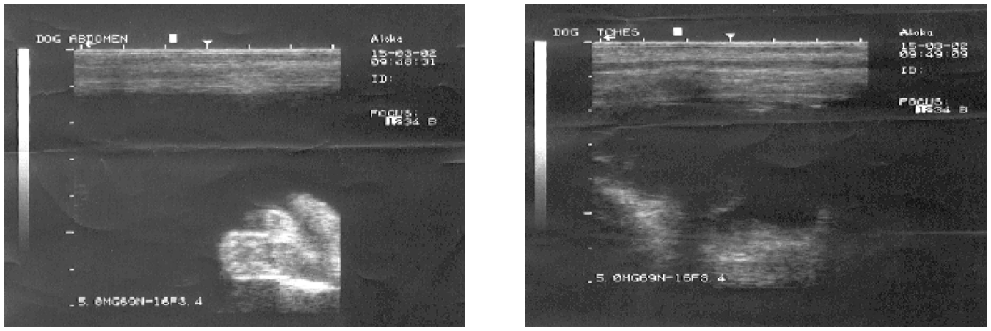

Fig. 2. Abdominal (left) and thoracic (right) ultrasonographies of a dog following intoxication with the anticoagulant rodenticide bromadiolone by day 1.

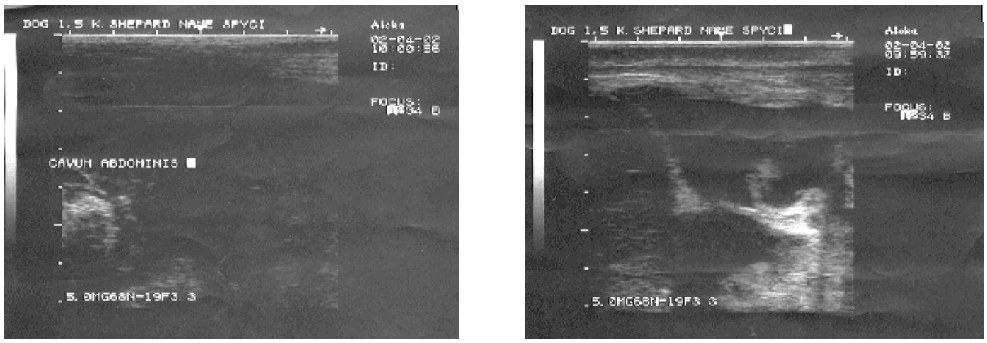

Fig. 3. Abdominal (left) and thoracic (right) ultrasonographies of a dog following intoxication with the anticoagulant rodenticide bromadiolone by day 12.

in the left intercostal spaces VII–VIII and the right intercostal spaces IV–V. The punctate was with an intensively red colour and odourless. The results of analysed punctates are presented in Table 1, showing that they were haemorrhagic transudates. Similar results showed the data for abdominal punctates.

The laboratory parameters, assessed during the hospitalization period (Table 2) showed a decreased Hgb concentration – 82 g/l, RBC counts – 5.19 T/l and Hct – 33.3% and increased WBC counts - 19.1 G/l. Until the end of the observation,

Table 1. Results from the analysis of thoracic punctates obtained from a dog following intoxication with the anticoagulant rodenticide bromadiolone

Parameters	Days after the hospitalization				
	1	3	6	9	12
Amount, mL	1700	500	650	400	250
Density	1.005	1.008	1.010	1.012	1.015
Protein, g%	2.0	2.5	2.5	3.0	3.0
Rivalta's test	-	-	±	±	±
Sediment	Plenty of erythrocytes. Polymorphonuclear leukocytes and endothel cells				

Table 2. Clinical laboratory blood examinations data of a dog following intoxication with the anticoagulant rodenticide bromadiolone

Parameters	Days after the hospitalization				
	1	3	6	9	12
Haematological parameters					
Haemoglobin, g/L	82	88	98	115	122
RBC, T/L	5.19	5.24	5.54	7.39	8.56
Haematocrit values, %	33.3	35.7	43.4	55.3	61.1
WBC, G/L	19.1	15.2	16.4	13.8	12.1
Differential WBC counts					
Eosinophils, %	0	0	1	1	0
Metamyelocytes, %	3	5	3	4	2
Band neutrophils, %	18	25	14	13	22
Segmented neutrophils, %	70	64	65	77	62
Lymphocytes, %	8	6	16	5	14
Monocytes, %	1	0	1	0	0
Biochemical parameters					
Blood sugar, mmol/L	6.48	6.23	6.87	5.54	4.82
Total bilirubin, μmol/L	28.6	22.8	23.5	18.1	13.2
ASAT, U/L	34.1	32.2	20.8	15.0	12.8
ALAT, U/L	65.2	54.8	44.2	35.7	40.1
Urea, mmol/L	18.8	12.4	9.6	12.8	10.2
Uric acid, μmol/L	78.8	64.2	96.5	118.4	128.1
Creatinine, μmol/L	148	124	109	111	94

those parameters returned to their physiological values. The differential WBC counts were suggestive for a neutrophilia with a regenerative shift to metamyelocytes (3–5%).

Higher blood levels were measured for blood sugar, bilirubin and ALAT. They remained elevated until the end of the hospitalization although a tendency towards normalization was observed. Significant deviations in the levels of ASAT, urea, creatinine, uric acid, ESR and coagulation liver function tests were not observed during the period of the study.

The mechanism of intoxication with dicoumarin anticoagulants is realized by a specific inhibition of blood coagulation via shunting of vitamin K from the enzymatic system that catalizes the formation of proconvertin, prothromin and other factors of blood coagulation in liver cells, resulting in prolongation of thrombin time (Nieuwenhuys *et al.*, 2000; Begent *et al.*, 2001). Blood vessels lose their elasticity

that results in a necropsy finding of large blood vessels ruptures, clinically manifested by massive haemorrhagies and haematomas (Shan and Suttie, 1095; Howe and Webster, 2000; Grainger *et al.*, 2001). Petterino and Paolo (2001) observed that anticoagulant rodenticides impair the cellular recirculation of vitamin K causing seconday coagulopathies. Another cause for haemocirculatory troubles is the calcification of large blood vessels (aorta, hepatic, mesenterial, femoral, renal etc. arteries) (Howe and Webster, 2000; Price *et al.*, 2001). Anticoagulants are shown to increase glomerular filtration rate (Druid *et al.*, 1999), clinically manifested by proteinuria.

Dicoumarin rodenticide bromadiolone inhibits blood coagulation and affects blood vessels via an analogous mechanism (Blocker and Roberts, 1999; Howe and Webster, 2000; Pettrino and Paolo, 2001). This is an explanation for the haemocirculatory disorders manifested by accumulation of haemorrhagic transudate in the thoracic (liquidothorax) and the abdominal cavity (ascites), observed by us. It could be presumed that the simultaneous decrease of RBC counts, haemoglobin (normochromic anemia) and haematocrit values occurred as a consequence of the blood loss. The observed leukocytosis with neutrophilia and regenerative shift to metamyelocytes most probably occurs as a result of the local inflammation following the irritant effect of the preparation on gastric and intestinal mucosa correlating with hyperthermia. The big amount of thoracic transudate is most likely responsible for the collapse of the compressed pulmonary parenchyma and the signs of respiratory insufficiency – polypnea, dyspnea, costo-abdominal type of breathing (Blocker and Roberts, 1999). The tachycardia is a compensatry mechanisms for oligochromaemia, erythropenia and pulmonary insufficiency. The prolongation of thrombin time occurs after the inhibition of the formation of proconvertin and thrombin in hepatocytes (Price *et al.*, 2001; Petterino and Paolo, 2001). The hyperglycaemia, bilirubinaemia and increased ALAT activity are other signs of the toxic effect of anticoagulant rodenticides on liver parenchyma (Zhou and Chan, 2001; Begent *et al.*, 2001). It could be accepted that the same toxicodynamics is valid for bromadiolone intoxication as well.

Throughout the hospitalization period (days 1-15), 300 mL 20% glucose, 5 mL 10% vitamin C, 5 mL 20% caffeine sodium benzoate and 10 mg vitamin K_3 (Kavitol, Lannacher Heilmittel-Lannach, Austria) were intravenously administered once daily. The medication therapy was extended by intramuscular administration of 12 mL Lincomycin-Spectinomycin 5/10 (50 mg lincomycin hydrochloride and 100 mg spectinomycin dihydrochloride in 1 mL injectable solution) (Alfasan-Woerden, Holland) every 24 hours for 10 days; 20 mg furosemide (in 2 mL inuectable solution) every 12 hours for 3 days, followed by oral application of 40 mg furosemide tablets, twice daily for 10 days and 5 g urotropin once daily for 7 days.

The punctions of thoracic and abdominal cavities were followed by intrathoracal or intraperitoneal administration of 10 mL vitamin AD_3E (vitamin A 15000 UI, vitamin D3 20000 UI and vitamin E 0.01 g), 1 mL gentamicin (40 mg/mL), 5 mL 1% novocain (1 g procaine hydrochloride in 100 mL, VetProm, Radomir, Bulgaria) and 1 mL dexamethasone (2 mg dexamethasone disodium phosphate in 1 mL; Alfasan-Woerden, Holland).

After a 3-week therapy, the clinical and the laboratory status of the patient

was within the reference ranges and the dog was discharged from the clinic as cured.

It could be concluded that: 1. The spontaneous intoxication with the anticoagulant rodenticide bromadiolone in a dog was manifested by changes in the clinical status such as arexia, polydipsia, hyperthermia, polypnea, dyspnea, tachycardia, pale conjunctives, liquidothorax and ascites; 2. The changes in the laboratory indices were as followed: oligochromaemia, erythropenia, leukocytosis with neutrophilia and regenerative shift, hyperglycaemia, increased ALAT activity and prolonged thrombin time.

REFERENCES

Angelov, G., N. Ibrishimov and S. Milashki, 1999. Clinical Laboratory Investigations in Veterinary Medicine. "Prof. Marin Drinov", Sofia.

Begent, L., A. Hill, G. Steventon, A. Hutt, C. Pallister and D. Cowell, 2001. Characterization and purification of the vitamin K1,2,3 epoxide reductases system from rat liver. *Journal of Pharmacy and Pharmacology*, **53**, No 4, 481–486.

Blocker, T. and B. Roberts, 1999. Acute tracheal obstruction associated with anticoagulant rodenticide intoxication in a dog. *Journal of Small Animal Practice*, **40**, No 12, 577–580.

Druid, H., J. Nilsson, L. Rammer and G. Skude, 1999. Effect of anticoagulation on renal function and protein excretion in experimental acute ischemic renal failure. *Renal Failure*, **21**, No 6, 647–657.

Grainger, D., N. McWillam, T. Baglin and C. Byrne, 2001. Suppressing thrombin generation is compatible with the development of atherosclerosis in mice. *Thrombosis Research*, **102**, No 1, 71–80.

Howe, A. and W. Webster, 2000. Warfarin exposure and calcification of the arterial system in the rat. *International Journal Experimental Pathology*, **81**, No 1, 51–56.

Lehmann, D.E., 2000. Enzymatic Shunting: resolving the acetaminophen-warfarin controversy. *Pharmacotherapy*, **20**, No 12, 1464–1468.

Maroni, M., C. Colosio. A. Ferioli and A. Fait, 2000. Biological monitoring of pesticide exposure: a review. *Toxicology*, **143**, No 1, 1–118.

Nieuwenhuys, C., M. Feijge, S. Beguin and J. Heemskerk, 2000. Monitoring hypocoagulant conditions in rat plasma: factors determining the endogenous thrombin potential of tissue factor-activated plasma. *Thrombosis and Haemostasis*, **84**, No 6, 1045–1051.

Petterino, C. and B. Paolo, 2001. Toxicology of various anticoagulant rodenticides in animals. *Veterinary and Human Toxicology*, **43**, No 6, 353–360.

Price, P., H. June, J. Buckley and M. Williamson, 2001. Osteoprotegerin inhibits artery calcification induced by warfarin and by vitamin D. *Arteriosclerosis, Thrombosis and Vascular Biology*, **21**, No 10, 1610–1616.

Sengalevich, G., T. Tonev and A. Nikolov, 1998. Manual for application of chemicals for plant protection. VSI, Plovdiv.

Shan, D. and J. Suttie, 1975. Vitamin K requiment and warfarin tolerance in hamsters. *Proceedings Social Experimental Biology and Medicine*, **150**, No 1, 26–128.

Smith S., S. Kraft, D. Lewis, S. Melethil and L. Freeman, 2000b. Pharmacodynamics of warfarin in cats. *Journal of Veterinary Pharmacology and Therapeutics*, **23**, No 6, 339–344.

Smith, S., S. Kraft, D. Lewis and L. Freeman, 2000a. Plasma pharmacokinetics of warfarin enantiomers in cats. *Journal of Veterinary Pharmacology and Therapeutics*, **23**, No 6, 329–337.

Stone, W., J. Oconiewski and J. Stedelin, 1999. Poisoning of wildlife with anticoagulant rodenticides in New York. *Journal of Wildlife Disease*, **35**, No 2, 187–193.

Zhou, S. and E. Chan, 2001. Effect of ubidecarenone on warfarin anticoagulation and pharmacokinetics of warfarin enantiomers in rats. *Drug Metabolism*, **18**, No 2, 99–122.

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