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CLINICAL AND EXPERIMENTAL INVESTIGATIONS ON CHRONIC INTOXICATION WITH THE ANTICOAGULANT RODENTICIDE BROMADIOLONE IN DOGS. I. CLINICAL AND HAEMATOLOGICAL STUDIES

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ABSTRACT
Experiments for testing the toxic effect of the anticoagulant rodenticide bromadiolone in dogs treated orally in granules at 0.18 mg daily (1/70 LD_{50} for dogs) were performed over 3 months. Before the trial's beginning (days –15 and 0) and at post treatment days 15, 30, 45, 60, 75 and 90, changes in the clinical status (rectal body temperature, heart and respiratory rates) and some haematological parameters (haemoglobin erythrocyte counts, platelet counts, leukocyte counts, haematocrit, prothrombin time and activated partial thromboplastin time) were determined. Thoracic and abdominal ultrasonography was parallelly performed. The intoxication was accompanied by hypothermia, tachycardia, polypnea, anorexia to arrhexia, somnolence, weakness, cough, pale mucous, dyspnea, (nasal bleending) epistaxis, subcutaneous haematomas after venipuncture, enlarged abdomen, abdominal paint, pericardial, peritoneal and pleural effusions, erythropaenia, oligochromaemia, reduced haematocrit, prolonged coagulation time

Key words: dogs, anticoagulant, rodenticides, bromadiolone, brodifacoum, floucumafen, clinical sings and haematological changea.

INTRODUCTION

Anticoagulant rodenticides are 4-hydroxycoumarin derivatives used for control of harmful rodent populations. They inhibit the cycling of vitamin K in the liver, interfering with blood coagulation and cause death from massive haemorrhages. Although used for control of rodents, they could induce intoxications in non-target animal species by ingestion of baits (primary toxicity) or by eating poisoned rodents (secondary toxicity) (1, 2). Secondary toxicity of anticoagulant rodenticides is reported in many animal species (birds and mammals) (3, 4, 5, 6, 7, 8, 9). Depending on their chemical structure, they are divided into first generation, synthesized and implemented in 1940–1960 (warfarin, pindone, coumatetraly etc.) and second generation, synthesized in 1970–1980 (difenacoum, bromadiolone, brodifacoum, flocoumafen etc.) (10). These compounds inhibit vitamin K-epoxide reductase in the liver, causing reduced synthesis of vitamin K-dependent coagulation factors II, VII, IX, and X (11, 12, 13, 14). Vitamin K is an essential cofactor of postribosomal carboxylation (activation) of blood coagulation factors, involved in extrinsic and intrinsic coagulation cascade. The lack of vitamin K-epoxide reductase disturbs the recycling of vitamin L and animals need new external sources of the vitamin. The intoxication with anticoagulant rodenticides depletes the stores of active vitamin K in the liver and stops the synthesis of coagulation factors (II, VII, IX, and X). After these factors are depleted, only the application of vitamin K_1 could correct the impaired haemostasis within 12–48 h (12, 13, 14). Second-generation anticoagulants are

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more toxic (100 to 1000 times) compared to first-generation ones. They possess a higher affinity to vitamin K cycle hepatic inhibition sites and a higher rate of accumulation and persistence in it (15, 16, 17, 18). The intoxication with anticoagulant rodenticides in dogs is frequently encountered (19).

Previous studies of ours in acute and subacute bromadiolone poisonings in dogs (20, 21) have established the following clinical and haematological changes: hypothermia, tachycardia, polypnea, pale conjunctivae in some animals, conjunctival haemorrhages and hyphaema in others, haemoptysis, rhinorrhagia, buccal and gingival bleeding, haematuria with dysuria, somnolence, prolonged bleeding after venipuncture with haematoma formation, peritoneal and pleural effusions of blood, erythropaenia, thrombocytopenia, oligochromaemia, reduced haematocrit, enhanced ESR, prolonged coagulation times (APTT and PT). These changes were similar to those observed in dogs, cats, and horses treated with brodifacoum and diphacinone (22, 12, 23, 13, 24, 25, 26, 27, 28).

Due to the rapid occurrence of toxic effects in acute intoxications with anticoagulant rodenticides, investigations over a longer period of time (chronic) were not carried out (29).

Having in mind the importance for spontaneous intoxications in stray dogs after ingestion of baits containing anticoagulant rodenticides, the aim of this study was to study the toxic effects of bromadiolone at minimum concentrations on clinical and haematological indices in dogs with regard to the timely diagnostics and treatment.

MATERIAL AND METHODS

Experiments were conducted with 6 mixed-breed dogs of both genders, weighing 12-14 kg, aged 4–5 years. One month before the experiment started, animals were housed in a room 5/4.5/5 m of size. They were treated twice against parasites with Cestal plus (Ceva, Santé Animale, France), 1 tablet per 10 kg b.w. at 14-day interval. Ectoparasites were controlled by Bolfo® Powder (Bayer, Germany). The anticoagulant rodenticide (Bromadiolone 0.25%) was ingested orally with dry food ("Strong Maintenance Menu) and drinking water was available ad libitum. The dry food granules were treated with 1/70 LD_{50} of bromadiolone for dogs (0.18 mg daily). Each dog received 350 g dry food per day. On days –15 and 0, as well as on post treatment days 15, 30, 45, 60, 75 and 90, the body temperature (^{0}C), heart and respiratory rates (min^{-1}), visible mucous coat colour, appetite, perception, urination, defecation etc. were determined by routine clinical diagnostic methods. After puncture of v. jugularis or v. cephalica antebrachii, blood for analysis was collected for determination of: haemoglobin (g/l), erythrocyte counts (T/l), platelet counts (G/l), haematocrit (%), on an automated analyzer BC 2800, VET, China. activated partial thromboplastin time (APTT, s) and prothrombin time (PT, s) were assessed with commercial kits (Hemostat TT, Human, Germany) on a coagulometer (Amelung-Coagulometer KC 1A). Ultrasonography was done with ultrasound equipment Chison 600 VET (China) with a convex 5MHz transducer.

Data were statistically processed by one-way ANOVA with Turkey-Kramer as post hoc test.

RESULTS

1. Clinical studies

Rectal body temperature of dogs before the experiment ranged within 38-39°C (38.3±0.1°C) day –15 before the intoxication and was 38.4±0.2°C by day 0. After feeding dogs dry food treated with bromadiolone, body temperature decreased by day 75 compared to control values – 37.5±0.08 °C ($p>0.05$ to $p<0.01$) and minimum values were attained at the end of the trial – 36.8±0.10 ($p<0.001$). In addition, heart and respiratory rates increased statistically significantly compared to controls (days –15 and 0) (75±3.3/min, 77±2.3/min and 25±1.5/min, 22±3.1/min, respectively) by the 75th day ($p<0.01$, $p<0.05$) and reached peak values on day 90: 102±6.3/min ($p<0.001$) and 42±2.4/min ($p<0.001$). During the intoxication course, other clinical signs were also observed: anorexia to arrhexia, pale mucous coats, weakness, somnolence, cough, dyspnea, enlarged abdomen, abdominal pain, nasal bleeding (epistaxis), subcutaneous haematomas after venipuncture. By means of ultrasound imaging, anechoic areas were observed in thoracic and abdominal cavities (haemothorax and haemoperitoneum), resulting from blood effusions **(Fig. 1 and 2).**

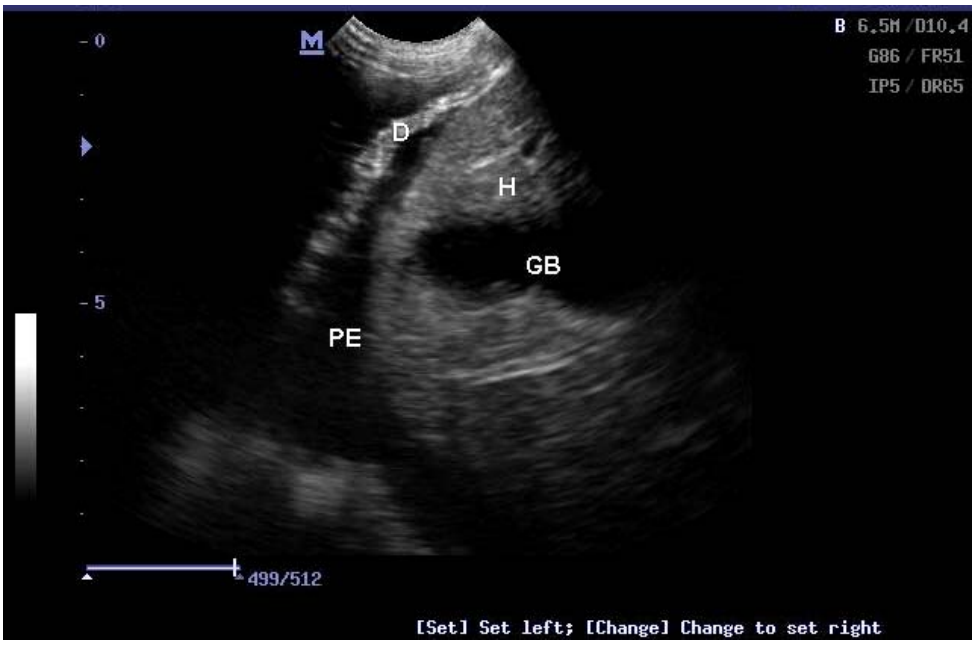

Fig. 1. Ultrasonogramme of peritoneal effusion in a dog, chronically intoxicated with bromadiolone. The transducer is positioned sagittally in the right subcostal space, oriented obliquely cranially. The liver (H), gall bladder (GB), diaphragm (D) and peritoneal effusion (PE) are visualised.

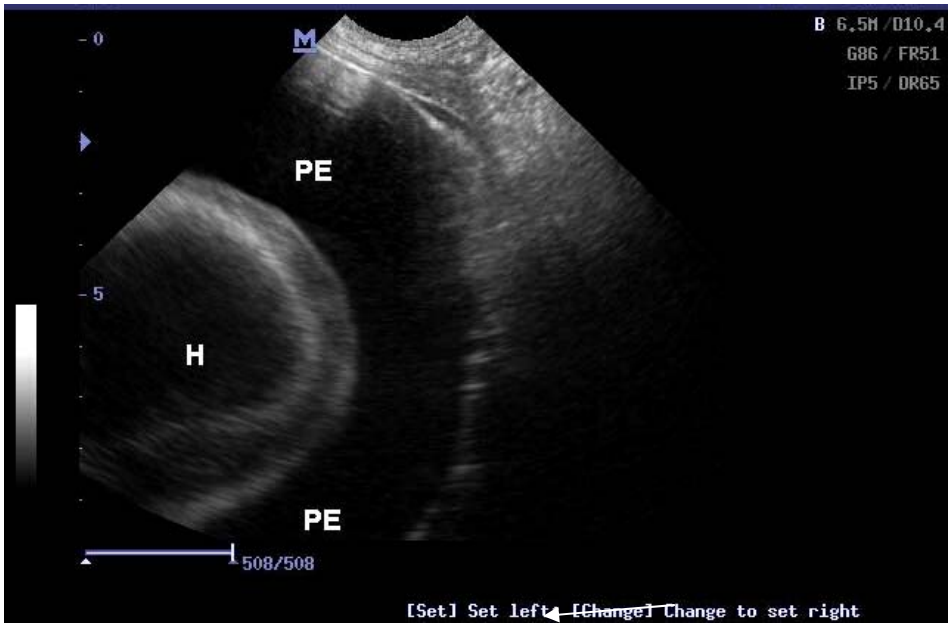

Fig. 2. Ultrasonogramme of pericardial effusion in a dog chronically intoxicated with bromadiolone. The transducer is positioned from the left side in the 6th intercostal space. A vast anechoic area (pericardial effusion; PE), between the heart (H) and the pericardium is observed (arrows).

2. Haematological studies

The haemoglobin content before the start of experiments was 168.5±2.44 g/l on day –15 and 170.3±3.21 g/l on day 0. After feeding dogs dry food treated with the tested anticoagulant rodenticide, it decreased by day 75 to 129±4.19 g/l ($p<0.001$). The oligochromaemia attained its peak by the end of the trial (day 90) – 97.5±7.6 g/l ($p<0.001$).

Red blood cell counts decreased from 6.75±0.48 T/l on day –15 and 6.56±0.37 T/l on day 0 to 4.07±0.22 T/l ($p<0.05$ to $p<0.01$) by the 75th day, and the lowest RBC counts were measured on day 90 – 2.58±0.41 ($p<0.001$).

Before feeding fogs dry food granules, treated with bromadiolone, haematocrit values were 45.1±2.33% (day –15) and 43.96±3.62 % (day 0). Lower values were registered on day 75 – 31.11±2.62 % ($p<0.05$). By the end of the study (90th day), haematocrit values were the lowest – 20.45±1.10% ($p<0.001$).

Platelet counts I the beginning were between 263.1±14.2 (day –15) and 254.5±15.8 (day 0). They decreased statistically significantly on the 75th day – 172±5.23 G/l ($p<0.001$) but lowest counts were attained by the 90th day 118±9.20 G/l ($p<0.001$).

Leukocyte counts were initially 11.63±0.88 G/l (day –15) and 11.38±0.51 G/l (day 0). After feeding bromadiolone-treated dry food, leukocyte counts increased to 16.08±0.57 G/l 75 days later ($p<0.05$). Maximum values were determined on day 90 – 20.2±0.9 G/l ($p<0.001$).

Coagulation parameters (APTT and PT) were prolonged by the 60th day – 51.7±5.14/s ($p<0.05$) and 53.21±3.59/s ($p<0.05$) respectively as compared to control values on days –15 and 0 (16.21±0.89/s, 16.56±1.16/s for APTT and 6.73±0.23/s, 6.50±0.14/s for PT). During the chronic poisoning with anticoagulant rodenticide bromadiolone, these coagulation times were prolonged until 184.33±17.63/s for APTT and 208.5±13.59/s за PT by the 90th day.

Table 1. *Changes in clinical status parameters (body temperature, heart rate and respiratory rate) in dogs fed granules treated with bromadiolone at a dose of 1/70 LD_{50} (0.18 mg per day). The different superscripts [a,b,c] within a row indicate statistically significant differences in a given parameter with time ($p<0.05$).*

Parameters	Days							
	-15	0	15	30	45	60	75	90
Body temperature, °C	38.3 0.1	38.4± 0.2	38.4± 0.1	38.5± 0.2	38.4± 0.2	38.1± 0.2	37.5± 0.08[b,b,c]	36.8± 0.1[c]
Heart rate, min^{-1}	75± 3.3	77± 2.3	78± 2.2	76± 3	77± 3.3	80± 3.8	97± 3.7[b,c]	102± 6.3[a,b,c]
Respiratory rate, min^{-1}	25± 1.5	22± 3.1	21± 3.5	22± 2.9	22± 2.1	25± 2.2	35± 1.8[a,b]	42± 2.4[b,c]

DISCUSSION

Accelerated difficult breathing is a compensatory mechanism of paraclinical changes observed by us (20, 21) and others (12, 28, 30, 31, 24, 25, 32, 33, 34, 35) – oligochromaemia, erythropaenia, reduced haematocrit, in dogs, cats, horses, men and wild ducks. Normochromic anaemia (oligochromaemia correlates with erythropaenia) and reduced haematocrit result from the multiple massive haemorrhages occurring due to the impaired blood coagulation (36, 37, 38, 39, 6, 7, 40, 41, 35), after the challenge with the tested rodenticide. The changes in the respiration could be attributed to the morphological changes in lungs (haemorrhages, congestive hyperaemia, interstitial oedema, swelling of bronchial and parabronchial mucosa, desquamation of epithelial cells in bronchioles) in dogs, lambs, birds and humans (42, 43, 44, 6, 34, 41, 45, 46, 47). On the other hand, these haematological changes decrease the oxidation of blood which is compensated by tachypnea. Hypothermia could be related to anaemia and brain haemorrhages (48, 12, 49, 50). The thrombocytopenia observed by us and other researchers (51, 12, 28, 13, 14, 52, 53, 54, 24,

25, 6, 33, 55) is related to impaired haemostasis. Leukocytosis could result from local inflammation caused by the irritating effect of the preparation on gastric and intestinal mucosae (12, 56, 34, 50). On the other side, leukocytosis could be due to enhanced release of neutrophils from bone marrow, secondary to haemorrhagic diathesis (57, 26).

Table 2. *Changes in some haematological parameters (Hb, Er, Hct, Plt, Lev, APTT and PT) in dogs fed granules treated with bromadiolone at a dose of 1/70 LD_{50} (0.18 mg per day). The different superscripts ^{a,b,c} within a row indicate statistically significant differences in a given parameter with time ($p<0.05$).*

Parameters	Days							
	-15	0	15	30	45	60	75	90
Hb (g/l)	168.5± 2.44	170.3± 3.21	172± 5.14	167.3± 7.41	164.6± 5.57	157± 6.52	129± 4.19^{b,c}	97.5± 7.6^{c}
Er (T/l)	6.75± 0.48	6.56± 0.37	6.39± 0.40	6.57± 0.55	6.43± 0.51	5.77± 0.42	4.07± 0.22^{a,b,c}	2.58± 0.41^{c}
Hct (%)	45.1± 2.33	43.96± 3.63	42.7± 2.55	45.11± 2.60	43± 2.93	40.4± 3.09	31.11± 2.62^{a,c}	20.45± 1.10^{c}
Plt (G/l)	263.1± 14.2	254.5± 15.8	240.8± 8.1	264.8± 10.4	253± 12	236± 5.36	172± 5.23^{c}	118± 9.20^{c}
Lev G/l	11.63± 0.88	11.38± 0.51	10.63± 0.92	11.8± 0.81	11.4± 0.70	16.00± 0.55^{a}	18.3± 1.00^{c}	21.2± 0.9^{c}
APTT (s)	16.21± 0.89	16.56± 1.16	19.7± 2.27	22.7± 1.79	28.9± 2.86	51.7± 5.14^{a}	136.26± 10.71^{c}	184.33± 17.64^{c}
PT (s)	6.73± 0.23	6.50± 0.14	7.51± 0.27	10.00± 0.59	24.41± 3.17	53.21± 3.59^{a}	132.28± 23.93^{c}	208.5± 13.59^{c}

Prolonged coagulation times (APTT and PT), due to challenge with brodifacoum, diphacinone and warfarin are reported in humans, dogs, horses, cats and birds (58, 59, 28, 60, 13, 35, 61, 62, 63, 24, 25, 26, 64, 33). The causes for these blood circulatory disorders are the inhibition of cell recycling of vitamin K_1 in the liver, induced by anticoagulant rodenticides (65, 66, 67). The latter are antagonists of vitamin K_1 epoxide reductase and thus, provoke depletion of vitamin K_1-dependent blood coagulation factors (II, V, VII, IX and X). They participate in intrinsic and extrinsic systems of coagulation cascade and thus, cause secondary coagulopathies clinically manifested with external and internal haemorrhages (12, 68, 13, 69, 14, 56, 24, 25, 41). The impaired

haemostasis is responsible for external and internal bleedings after injury. Death occurs approximately 3 to 10 days after ingestion of baits in acute intoxications (70). The prolongation of prothrombin time by 25% or more is an indicator of intoxication with anticoagulant rodenticides (40). Bachman and Salivan (1983) (36) presume that dose-dependent prolongation of prothrombin time in rats after treatment with brodifacoum could be partly attributed to enterohepatic circulation of the compound, i.e. its slower elimination from the liver.

Coumarin rodenticides are metabolized in the liver and eliminated through the gall bladder. The incessant transfer from blood circulation into the liver sustains high liver concentrations of anticoagulant rodenticides while blood levels simultaneously decrease. Prothrombin time is use as an early parameter in intoxications with anticoagulant rodenticides in domestic mammals (23) and is checked on a regular basis to control coumarin therapy in men (72). The observed by us and other authors (13, 49, 24, 25) clinical signs, pleural and peritoneal blood effusions (24, 25, 4, 71, 72, 52) are a functional response to impaired blood coagulation mechanisms.

CONCLUSION

In conclusion, it could be summarised that the chronic intoxication with the anticoagulant rodenticide bromadiolone (0.25%) in dogs at a dose equal to 1/70 LD_{50}, oral dose for dogs, provoked changes in the clinical status (hypothermia, tachycardia, polypnea, anorexia to arrhexia, pale visible mucous coats, weakness, cough, dyspnea, somnolence, abdominal distention, abdominal pain, epistaxis), subcutaneous haematomas after venipuncture. The clinical picture is further complicated by pericardial, pleural and peritoneal effusions of blood. The haemorrhagic diathesis provoked by the tested dose of the preparation resulted from the impaired blood coagulation mechanism. The changes in the red and white blood pictures (erythropaenia, oligochromaemia, reduced haematocrit, leukocytosis, prolonged coagulation times) result from the toxic effect of bromadiolone. The clinical and paraclinical changes could be used in the diagnostics of chronic bromadiolone intoxications in dogs and for monitoring of antidote treatment.

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