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Hematological Profile and Aminotransferase Activity in Kintamani Bali Puppies Injected with High Doses of Ivermectin

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ABSTRACT

Ivermectin toxicity is known to cause harmful side effects or even death in dogs intolerant to the medication. Intolerant dogs have a mutation in the MDR-1 (Multi-Drug Resistance) gene, so they lack the P-glycoprotein gene that removes drugs from the brain. Therefore, this study aimed to determine ivermectin toxicity in Kintamani Bali puppies by examining physiological responses based on hematological profiles and aminotransferase activity after a high-dose injection. A laboratory observational approach was used, and the samples were 25 healthy female Kintamani puppies based on a veterinary examination, aged 3-6 months, weighing 6.32 ± 1.18 kg, randomly divided equally into five treatment groups. The treatments included a placebo (1ml Aqua Pro Injection) as a control, as well as a single dose of ivermectin injection sequentially 200, 400, 800, and 1600 µg/kg subcutaneously. Blood samples were collected before treatment and after 7 and 14 days post-treatment. The hematologic parameters observed included levels of hemoglobin, erythrocytes, hematocrit, total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, and basophils, as well as blood biochemistry, namely aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. Observation results after 4 hours of administration of ivermectin at doses of 800 and 1600 µg/kg of puppies showed changes in behavior, restlessness, depression, tremors, mydriasis, hypersalivation, anorexia, and polydipsia. Meanwhile, the results of hematological examination on the seventh day after ivermectin treatment showed a trend of erythropenia, leukocytosis, a decrease in hemoglobin levels, and an increase in aminotransferase enzyme activity. This condition continued until day 14, but the physiological parameter values showed that the puppy's condition gradually improved compared to the seventh day after treatment. There were significant differences in the blood profile, AST, and ALT of Kintamani puppies injected with ivermectin at doses of 800 and 1,600 ug/kg compared to controls on days 7 and 14 after and before treatment. It was concluded that high-dose ivermectin injections in Kintamani Bali puppies caused toxicity with clinical signs of erythropenia, decreased hemoglobin, leukocytosis, and increased aminotransferase activity.

Keywords: Aminotransferase, Blood profile, Ivermectin, Kintamani dogs, Toxicity

INTRODUCTION

Kintamani Bali dogs, a native Indonesian breed, are well-known for their attractive appearance, medium size, trainability, and loyalty, culminating in frequent adoption among dog enthusiasts (Putra and Darmayanthi, 2023). The constantly growing number of enthusiasts has also led to an increase in their population. However, a high population density without a balanced environment poses risks of viral, bacterial, and parasitic diseases (Everman et al., 2011; Short et al., 2017). Parasitic diseases can be attributed to both endoparasite and ectoparasite infestations, with the most commonly reported endoparasitic diseases in dogs being infestations with worms such as *Toxocara canis*, *Ancylostoma caninum*, *Trichuris vulvis*, *Dipylidium caninum*, and *Necator sp*. (Pesavento and Murphy, 2014). Meanwhile, frequently mentioned ectoparasites include the *Rhipicephallus sanguineus* tick (Dantas-Torres, 2010), *Heterodoxus spiniger* and *Thrichodectes canis* fleas, *Ctenocephalides (C.) felis*, and *C. canis* lice (Rinaldi et al., 2007), as well as the *Sarcoptes scabei* and *Demodex canis* mites (Vladimirovna Moskvina, 2017). Parasitic infections require serious attention due to their ability to cause a deteriorated appearance in dogs and their zoonotic nature (Chomel, 2014; O'Neil, 2018).

Nematode worm infections in Kintamani Bali dogs have been reported in their natural habitat (Evayana et al., 2017). Prevention and management of worm infestations require specific medication tailored to the diagnosis. Deworming is generally performed by administering prescribed drugs every 3-4 months regularly. Some common deworming drug options include albendazole, febendazole, pyrantel pamoate, and praziquantel. As for the management of ectoparasite cases, it is accomplished topically using shampoos, sprays, anti-parasitic creams, or by administering oral medications (Plumb, 2008; Riviere and Papich, 2018). This method is considered less effective as it requires a longer duration and the diligence of the dog owner in care (Riviere and Papich, 2018). Systemic drugs capable of addressing

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both endoparasites and ectoparasites include ivermectin and fipronil. Ivermectin is a macrocyclic lactone compound belonging to the chemotherapeutic class. This active compound, often referred to as endectocide, is produced from the fungus *Streptomyces avermitilis* and has a broad spectrum of action (Prichard et al., 2012; Campbell, 2016; Lotfalizadeh et al., 2022).

Ivermectin toxicity has been reported to have a negative impact on the host animal by causing damage to various organs such as the liver, kidneys, brain, reproductive organs, and endocrine glands (El-Saber Batiha et al., 2020). Several studies report that ivermectin has induced nephrotoxicity in rats, rabbits, mice, and dogs (Al-Jassim et al., 2016; Wilson Magdy et al., 2016; Dey et al., 2017). Ivermectin is lipophilic, with high concentrations in the liver causing oxidative stress and hepatotoxicity (Zhu et al., 2013; Celis-Giraldo et al., 2020). Neurotoxicity events are associated with P-glycoprotein deficiency (Kiki-Mvouaka et al., 2010; Merola and Eubig, 2018), so ivermectin accumulates in the brain, causing damage and dysfunction of the cerebral cortex (Bates, 2020). According to previous studies, some dog breeds such as collies, Australian shepherds, Shetland sheepdogs, old English sheepdogs, longhaired whippets, German shepherds, and some mixes of these breeds are intolerant to ivermectin, leading to acute toxicity and death (Merola and Eubig, 2018). Various clinical symptoms of intolerance are associated with neurotoxicity, namely depression, hypersalivation, tremors, mydriasis, ataxia, seizures, coma, and ultimately death (Yas-Natan et al., 2003; Bates, 2020). The recommended dosage for oral use is 100-600 µg/kg, while the dosage for subcutaneous injection is 200-400 µg/kg body weight, depending on the diagnosis and therapeutic purpose (Plumb, 2008; Riviere and Papich, 2018). The use of macrocyclic lactone drugs, including ivermectin, has shown resistance in endoparasites (Yanuartono et al., 2020). Field observations found that a high dose of 1.000-2.000 µg/kg of ivermectin therapy practices exceed the therapeutic dose, posing toxic risks (Unpublish data). Therefore, this study aimed to find the possible toxicity of high doses of ivermectin in Kintamani Bali puppies by assessing behavioral changes, hematological profiles, and aminotransferase activity.

MATERIALS AND METHODS

Ethics approval

The Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University, Badung, Indonesia, gave ethical approval for this research with certificate number B/247/UN14.2.9/PT.01.04/2021.

Experimental animal

The Kintamani Bali puppies were obtained from Sukawana Village, Kintamani Subdistrict, Bangli Regency, Bali, Indonesia, as the native habitat. Purposive sampling was used to select healthy female puppies by veterinarian, aged 3-6 months, body weight 6.32±1.18 kg, and subsequently acclimatized for a minimum of 14 days in the experimental facility. This research was carried out from September to November 2022 at the Veterinary Pharmacy and Pharmacology Laboratory, Faculty of Veterinary Medicine, Udayana University, Indonesia. This was accomplished to adapt the test animals to the new environment and ease the handling by the study team. The experimental animals were placed in individual cages equipped with food and water containers. Commercial feed and fresh drinking water were provided *ad libitum*. The experimental room was maintained at a temperature ranging from 25 to 27°C, 60-70% humidity, and a 12-hour light-dark cycle.

Study design

This study used a complete randomized design with a laboratory observational analysis setting. A total of 25 Kintamani Bali puppies were randomly divided into five treatment groups, each having five replications. Ivermectin 1% (Intermectin, manufactured by Interchemie werken “De Adelaar” B.V. The Netherlands) was administered as a single dose, referring to the therapeutic dosage of 200-400 µg/kg (Plumb, 2008). In this study, the therapeutic dose was doubled for P3 and P4 groups. The treatments were as follows, P0 as the control group received a 1 ml placebo using free pyrogen aqua dest (Aqua Pro Injection, PT. Ikapharmindo Putramas, Indonesia) while P1, P2, P3, and P4 were each given ivermectin at doses of 200, 400, 800, and 1,600 µg/kg body weight through a single subcutaneous injection. Changes in the behavior of puppies were observed during the first three days, including depression, hypersalivation, mydriasis, tremors, ataxia, seizures, appetite, and drinking. Subsequently, blood samples were collected for hematological profile (Hemoglobin, erythrocytes, hematocrit, leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, and basophils) and aminotransferase activity examinations before treatment, and on day 7, and 14 days after ivermectin injection.

Blood collection

Dog blood collection was carried out aseptically through the cephalic vein using a venoject. In this regard, 1 ml was collected into a blood vacutainer with Ethylenediamine Tetra-acetic Acid (EDTA) for routine blood examinations

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and 2 ml into a vacutainer without anticoagulant for blood biochemical examinations. The blood samples were taken to the laboratory using a coolbox provided with ice gel cooling for further examination.

Hematology and blood chemistry examination

Routine blood tests were performed using the LICARE 3-Part Vet Auto Hematology Analyzer (Licare Biomedical Limited, China). Parameters observed included hemoglobin levels, erythrocytes, hematocrit, total leukocytes, and leukocyte differential counts. The Seamaty Veterinary Automatic Biochemical Analyzer (Chengdu Seamaty Technology Co., Ltd, China) was used for blood chemistry examination. The observed parameters included alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. ALT and AST examination using the Seamaty Vet Chemistry Reagent kit (Chengdu Seamaty Technology Co., Ltd, China). Seamaty veterinary reagent disc adopts microfluidic technology. It contains optical and mechanical components, which can be used with the instrument to participate in each stage of blood analysis.

Statistical analysis

Research data includes erythrocytes, hemoglobin, hematocrit, leukocytes, and differential of leukocytes, AST, and ALT were presented in mean and standard deviation. Then, the research data was analyzed using the ANOVA and Duncan tests using the IBM SPSS Statistics version 26 device for Windows. If the P value is below 0.05, it is declared statistically significant ($p < 0.05$).

RESULTS AND DISCUSSION

Results

Observations of changes in the behavior of Kintamani Bali puppies aged 3-6 months after subcutaneous injection of various doses of ivermectin are summarized in Table 1. All puppies felt pain, grumbling, restlessness, and feelings of fear immediately after the ivermectin injection treatment. Behavioral changes indicating toxicity were found in the group receiving high doses of ivermectin (800 and 1,600 µg/kg), including restlessness, depression, tremors, mydriasis, hypersalivation, anorexia, and polydipsia. No clinical signs of ataxia or seizures were found in all treatment groups. Visually, it appears that higher doses show stronger changes.

The results of the blood examination of Kintamani puppies are presented in Table 2. As can be seen in the table, total erythrocytes, hemoglobin, and hematocrit decreased significantly ($p < 0.05$). Total leukocytes and monocytes increased significantly in animals treated with high doses of ivermectin compared to the control group ($p < 0.05$). These significant changes occurred on 7 and 14 days after treatment compared to the first day before treatment.

Table 3 summarizes measurements of the aminotransferase enzyme activity of Kintamani Bali puppies, which appeared to increase after injection of high doses of ivermectin. AST and ALT enzyme activities increased significantly 7 and 14 days after treated ivermectin, compared to the control group and the first day before treatment ($p < 0.05$).

Table 1. Behavior change of Kintamani puppies aged 3-6 months after being given different doses (200, 400, 800, and 1600 µg/kg) of ivermectin

Behavioral changes	Control	Dose of Ivermectin (µg/kg)				Explanation
		200	400	800	1600	
Restlessness	No	Yes	Yes	Yes	Yes	All puppies exhibited pain, grunted, and became restless immediately after receiving a subcutaneous injection of ivermectin.
Depression	No	No	No	Yes	Yes	Puppies show anxiety and seem to want to avoid being held while feeding. This behavior began to appear after 12 hours of ivermectin injection.
Hypersalivation	No	No	No	Yes	Yes	The puppy appears to be producing more saliva than usual.
Mydriatic	No	No	Yes	Yes	Yes	Pupils were measured after 4 hours of ivermectin injection, showing that mydriasis occurred compared to pupil size before and after treatment.
Tremor	No	No	No	No	Yes	Tremors were observed after 4 hours of subcutaneous injection of ivermectin at a dose of 1,600 µg/kg, and disappeared after the third day of treatment.
Ataxia	No	No	No	No	No	None of the pups showed movement incoordination after ivermectin injection.
Seizures	No	No	No	No	No	None of the puppies had seizures
Appetite	Normal	Normal	Normal	Anorexia	Anorexia	Puppies show nausea and reduced appetite.
Drinking	Normal	Normal	Normal	Polydipsia	Polydipsia	There was an increased water consumption in puppies treated with ivermectin at doses of 800 and 1.600 µg/kg subcutaneously.

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Table 2. Hematological profile of Kintamani puppies aged 3-6 months after subcutaneous injection of different doses (200, 400, 800, and 1600 µg/kg) of ivermectin

Parameter	Day	Control (Placebo)	Dose of ivermectin (µg/kg)			
			200	400	800	1600
Erythrocyte (10^6/µL)	day 1	6.48 ± 0.70	6.99 ± 1.23	7.12 ± 1.48	6.81 ± 1.72^{A}	6.83 ± 1.52^{A}
	day 7	6.47 ± 0.71^{b}	6.65 ± 1.11^{b}	6.69 ± 1.32^{b}	6.22 ± 1.56^{Ba}	6.08 ± 0.88^{Ca}
	day 14	6.54 ± 0.77^{b}	6.80 ± 1.12^{b}	6.94 ± 1.35^{b}	6.47 ± 1.30^{Bab}	6.21 ± 0.92^{Ba}
Hemoglobin (g/dL)	day 1	14.12 ± 2.17	13.96 ± 2.11	14.02 ± 2.50	14.28 ± 2.25^{A}	14.02 ± 2.90^{A}
	day 7	14.13 ± 2.18^{c}	13.52 ± 1.90^{bc}	13.10 ± 2.62^{b}	12.78 ± 1.87^{Bb}	11.66 ± 1.19^{Ba}
	day 14	14.02 ± 1.96^{c}	13.88 ± 2.07^{c}	13.62 ± 2.41^{c}	12.86 ± 1.79^{Bb}	12.02 ± 1.83^{Ba}
Hematocrit (%)	day 1	42.62 ± 6.48	42.54 ± 7.79	42.16 ± 7.06	44.48 ± 9.63^{A}	43.02 ± 6.49^{A}
	day 7	42.04 ± 7.11^{b}	41.70 ± 7.25^{b}	40.24 ± 5.90^{b}	37.62 ± 3.71^{Bab}	34.70 ± 4.17^{Ba}
	day 14	43.24 ± 8.01^{b}	42.30 ± 7.68^{b}	41.58 ± 6.40^{b}	37.46 ± 3.42^{Bab}	35.36 ± 4.37 ^{Ba}
Leucocyte (10^3/µL)	day 1	15.16 ± 2.02	15.75 ± 1.88	16.26 ± 3.28	15.28 ± 3.07^{A}	15.06 ± 3.09^{A}
	day 7	15.15 ± 2.06^{a}	15.67 ± 1.69^{a}	16.05 ± 3.07^{ab}	17.29 ± 2.63^{Bb}	19.02 ± 3.11^{Cb}
	day 14	15.36 ± 3.05^{a}	16.03 ± 1.91^{ab}	16.16 ± 3.05 ^{ab}	17.71 ± 2.18^{Bb}	18.61 ± 2.85^{BCb}
Neutrophil (%)	day 1	54.90 ± 4.77	50.32 ± 6.10	49.66 ± 7.90	51.06 ± 8.43	49.62 ± 5.38
	day 7	53.04 ± 3.70	49.62 ± 6.17	48.26 ± 6.69	48.14 ± 9.33	45.96 ± 4.11
	day 14	52.64 ± 9.33	50.14 ± 5.90	48.52 ± 5.13	47.48 ± 4.38	47.10 ± 5.02
Lymphocyte (%)	day 1	36.14 ± 3.50	41.10 ± 6.53	40.76 ± 6.77	39.70 ± 7.18	39.02 ± 4.34
	day 7	37.88 ± 2.16	41.14 ± 6.19	41.96 ± 6.29	40.80 ± 8.22	41.26 ± 6.10
	day 14	38.28 ± 2.55	40.90 ± 6.23	41.56 ± 4.52	40.82 ± 3.77	41.14 ± 3.33
Monocyte (%)	day 1	4.94 ± 0.79	4.92 ± 1.14	5.84 ± 2.22	5.12 ± 1.47^{A}	6.53 ± 1.92^{A}
	day 7	4.92 ± 0.79^{a}	5.14 ± 1.30^{a}	6.04 ± 1.88^{ab}	6.94 ± 2.15^{ABb}	8.24 ± 1.20^{Bc}
	day 14	4.88 ± 0.75^{a}	5.12 ± 1.33^{a}	6.24 ± 1.61^{ab}	7.14 ± 1.98^{Bb}	7.68 ± 0.79^{Abb}
Eosinophil (%)	day 1	3.30 ± 1.68	3.03 ± 1.65	3.16 ± 1.09	3.52 ± 1.27	4.10 ± 1.39
	day 7	3.34 ± 1.70	3.26 ± 1.48	3.12 ± 1.09	3.32 ± 1.11	3.62 ± 0.97
	day 14	3.34 ± 1.70	3.06 ± 1.68	2.84 ± 1.06	3.30 ± 1.14	3.26 ± 1.11
Basophil (%)	day 1	0.72 ± 0.47	0.64 ± 0.49	0.58 ± 0.47	0.60 ± 0.41	0.71 ± 0.47
	day 7	0.82 ± 0.48	0.84 ± 0.30	0.62 ± 0.44	0.80 ± 0.40	0.92 ± 0.33
	day 14	0.86 ± 0.44	0.78 ± 0.38	0.84 ± 0.37	0.96 ± 0.34	0.82 ± 0.31

^{abc} Different superscript letters towards each row indicate significant differences ($p < 0.05$), ^{ABC} Different superscript letters towards the same column indicate significant differences ($P<0.05$)

Table 3. Enzymes activity of Kintamani puppies aged 3-6 months given different doses (200, 400, 800, and 1600 µg/kg) of ivermectin

Parameter	Day	Control (Placebo)	Dose of ivermectin (µg/kg)			
			200	400	800	1600
AST (µ/L)	day 1	38.62 ± 5.50	38.40 ± 7.64	36.20 ± 5.76	35.20 ± 8.58^{A}	33.80 ± 8.44^{A}
	day 7	38.80 ± 5.45^{a}	38.20 ± 6.76^{a}	42.20 ± 5.85^{a}	63.80 ± 12.83^{Bab}	113.20 ± 13.68^{Cb}
	day 14	37.40 ± 5.55^{a}	43.60 ± 5.32^{a}	43.00 ± 6.08^{a}	60.80 ± 12.48^{Bab}	82.80 ± 10.83^{BCb}
ALT (µ/L)	day 1	22.80 ± 3.42	27.60 ± 5.08	26.60 ± 6.69	28.80 ± 6.30^{A}	27.20 ± 4.44^{A}
	day 7	23.60 ± 3.05^{a}	29.02 ± 7.22^{a}	40.60 ± 7.54^{ab}	67.40 ± 8.26^{Bb}	108.20 ± 15.12^{Cc}
	day 14	23.20 ± 3.11^{a}	33.00 ± 5.70^{a}	34.20 ± 7.16^{a}	46.60 ± 7.64^{Bbc}	69.80 ± 12.23^{Bc}

^{abc}Different superscript letters towards the row indicate significant differences ($p < 0.05$); ^{ABC}Different superscripts letters towards the same column indicate significant differences ($P<0.05$), AST: Aspartat aminotransferase, ALT: Alanin aminotransferase

DISCUSSION

Ivermectin can cause harmful side effects when administered above the maximum dose or given to non-target animals (Siroka and Svobodova, 2013). Cases of ivermectin toxicity have been reported in pigs, cows, dogs, cats, horses, and turtles (Jourdan et al., 2015; Dey et al., 2017; Celis-Giraldo et al., 2020). In this study, clinical observations of behavioral changes were conducted before and after treatment with high doses of ivermectin up to the third day. Kintamani puppies were found to exhibit toxicity symptoms after treatment, including restlessness, depression, tremors, mydriasis,

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hypersalivation, anorexia, and polydipsia (Tabel 1). These symptoms are similar to clinical ivermectin poisoning findings reported in dogs (Hopper et al., 2002; Epstein and Hollingsworth, 2013), calves (Patel et al., 2018), horses (Norman et al., 2012), lions (Saqib et al., 2015), and rabbit (Branco et al., 2021). Until now, ivermectin is still the drug of choice in parasite control practices because of its broad spectrum of action. However, caution in its use is necessary, considering that this drug works well against parasites and has an impact on the host animal. Side effects found in various animals include nephrotoxicity, hepatotoxicity, neurotoxicity and reproductive toxicity (Salman et al., 2022).

The hematological profile of Kintamani puppies given high-dose ivermectin subcutaneously significantly differed from the control and therapeutic dose groups (200-400 µg/kg). Kintamani puppies experienced erythropenia, leukocytosis, and decreased hemoglobin after injection of ivermectin 2-4 times more than the recommended dose. These results are in line with Salman et al. (2022), who reported that male albino rats treated with Abamectin experienced a decrease in total red blood cells, an increase in total white blood cells, a decrease in hemoglobin, changes in serum enzyme levels, and a decrease in the number and motility of spermatozoa. Erythropenia and decreased hemoglobin presumably resulted from damage to the blood cells due to the presence of ivermectin in the bloodstream. The decrease in total erythrocytes can be caused by hemorrhage, hemolysis, low production, or other factors such as nutrition (Maglaras et al., 2017; Martinez et al., 2019). Deficiency in folic acid and Vitamin B12 impairs erythrocyte maturation during erythropoiesis, resulting in a decrease in their number in the bloodstream (Widyanti et al., 2018). Meanwhile, leukocytosis refers to increased leukocytes, typically found in patients with infections, inflammation, tissue necrosis, or leukemic neoplasia. Two researchers have reported hemorrhage in experimental mice after administration of ivermectin and Abamectin. A single injection of 50 mg/kg dose of ivermectin causes hepatic congestion and hemorrhage accompanied by centrilobular necrosis (Dadarkar et al., 2007). Oral administration of Abamectin, 2 mg/kg for 5 days, causes edema, hemorrhage, inflammatory cell infiltration, and tubular necrosis (Abdel-Daim and Abdellatief, 2018). Meanwhile, subcutaneous injection of ivermectin in rabbits at a dose of 0.4 mg/kg and in goats at 2 mg/kg causes congested blood vessels, tubular degeneration, desquamation and necrosis of tubular epithelium, glomerular necrosis, and infiltration of leucocyte (GabAllh et al., 2017). The same oxidative damage allegedly occurred in the puppies in this study, resulting in erythropenia and a significant decrease in hemoglobin. Tissue damage increases autophagy to clear damaged organelles and proteins formed by damaged hepatocytes (Zhu et al., 2013). This autophagy process involves many white blood cells, leading to inflammatory cell infiltration and leukocytosis in the animal (Salman et al., 2022).

The biochemical blood tests showed a significant increase in aminotransferase enzymes in the group administered a high dose of ivermectin. The enzymes reflect the integrity or integration of liver cells, and a significantly high increase may reflect the level of damage. The high activity in this study was due to increased drug metabolism in the liver, leading to hepatocyte damage (Senior, 2012). Ivermectin is highly lipophilic and can be distributed with a wide volume and induce oxidative stress (Salman et al., 2022). This leads to the accumulation of fat tissues, serving as a drug reservoir. The highest accumulation of ivermectin was found in the liver and fat tissues, while the lowest was detected in the brain and bone marrow (Zemkova et al., 2014; Juarez et al., 2018).

Ivermectin works by releasing and binding to Gamma-Aminobutyric Acid (GABA) neurotransmitters, which block nerve impulse transmission at the peripheral endings and smooth muscle cells of parasites, resulting in paralysis and death (Canga et al., 2009; Eraslan et al., 2010). The affinity for Glutamate-gated Chloride channel receptors at certain neuron synapses increases chloride ion permeability, leading to decreased appetite, fecundity, and parasite movement due to paralysis (Wolstenholme, 2011; El-Saber Batiha et al., 2020). Ivermectin metabolism occurs in the liver and is primarily excreted through feces, with a small portion released through urine. According to a previous study, several dog breeds are intolerant to ivermectin, including Collies, Australian Shepherds, as well as Shetland and Old English Sheepdogs (Merola and Eubig, 2018). These intolerant dogs have genetic abnormalities in the MDR-1 (multi-drug resistance) gene, resulting in a deficiency of P-glycoprotein (Kiki-Mvouaka et al., 2010; Mueller et al., 2020). This protein is responsible for removing residual drugs and their metabolites from the central nervous system (Riviere and Papich, 2018). Accumulation of ivermectin in the brain potentially causes acute toxicity and various disorders in the central nervous, gastrointestinal, and cardiovascular systems, which leads to an increased risk of death (Merola and Eubig, 2018).

The recommended therapeutic dose of ivermectin falls in the range of 200-400 µg/kg (Plumb, 2008; Martin et al., 2021). Associated toxicity in dogs without the MDR-1 gene mutation was reported with an acute oral LD50 of 8000 µg/kg (Plumb, 2008), while those with the MDR-1 gene defect have an acute oral LD50 of 200 µg/kg (Plumb, 2008; Woodward, 2012). Ruminant livestock farmers often increase the dose to tenfold the recommended level or about 2000 µg/kg due to a lack of understanding regarding the effective dosage (Yanuartono et al., 2020). Other researchers reported the LD50 of a single dose of ivermectin injection in rats at a dose of 50 mg/kg (Dadarkar et al., 2007). Furthermore, young animals are more sensitive to ivermectin toxicity than adults (Patel et al., 2018). In this study, indications of toxicity in puppies were shown by the increase in aminotransferase activity at a dose of 800-1600 µg/kg starting on day 7, and the levels remained high after 14 days of administration. It also indicated that the pharmacodynamics of high-dose ivermectin led to a tendency for erythropenia, leukocytosis, and a decrease in hemoglobin levels (Johnson-Arbor, 2022).

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CONCLUSION

In conclusion, subcutaneous injection of high doses of ivermectin (800-1.600 µg/kg) in Kintamani Bali puppies led to toxicity with clinical signs of erythropenia, leukocytosis, a decrease in hemoglobin levels, and an increase in aminotransferase activity. The results of the present study can serve as a reference for veterinary practitioners to properly administer ivermectin therapy to prevent toxicity and resistance.

DECLARATIONS

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Availability of data and materials

The authors confirm that the data presented is currently available upon reasonable request.

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Authors' contribution

Luh Made Sudimartini conceptualized and designed the study. Luh Made Sudimartini, Romy Muhammad Dary Mufa, and I Made Merdana conducted the study and collected and analyzed data. Luh Made Sudimartini wrote the manuscript. I Made Merdana provided advice and a scientific review. All authors checked and confirmed the last edition of the article.

Competing interests

The authors declare that there are no competing interests in the writing and processing of this article.

Ethical consideration

All the authors have checked ethical issues such as the plagiarism index, double publication, and any important publication ethics before the submission of this article.

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