Original Article

Exploring Breed-Related Patterns and Diagnostic Factors in Canine Hypothyroidism: Unveiling Novel Paradigms

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Abstract

Canine primary hypothyroidism is one of the most common endocrinopathy diseases in dogs affecting various body systems. In veterinary practice, the measurement of total thyroxine together with canine thyroid-stimulating hormone (TSH) was suggested to have high accuracy for diagnosing this disease. However, the changes in these recommended diagnostic parameters can be distinguished only in a hypothyroid dog with progressive deterioration or later in the disease. This study investigated the relevant predicting factors of canine primary hypothyroidism from animal characteristics and blood chemistry. Among the 672 dogs that met the inclusion criteria, 77 dogs had hypothyroidism and 179 had non-hypothyroidism. Age at diagnosis was significantly lower in the hypothyroid group (P = 0.01). Packed cell volume and serum albumin were significantly lower, while serum cholesterol and serum creatinine were significantly higher in the hypothyroid group (P < 0.001). Thai Bangkaew dogs had the highest risk for hypothyroidism (31.2%). Multivariable logistic regression identified 5 risk factors. Dogs of the Thai Bangkaew breed with skin lesions, anemia, hypercholesterolemia, and hypertriglyceridemia had 4,272 times greater risk of canine hypothyroidism. The potential association of risk factors, including anemia, hypercholesterolemia, hypertriglyceridemia, and skin lesions, suggests a strong diagnostic characteristic for canine hypothyroidism. Evaluation of serum cholesterol and triglyceride in health programs for Thai Bangkaew dogs would be advisable before investigating the thyroid profile, indicating that it is a common genetic risk factor (OR 8.21, [95% CI, 2.21-30.45]) in a variety of breeds.

Keywords: Canine primary hypothyroidism, Thai Bangkaew dogs, Risk factors

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Introduction

Hypothyroidism is one of the most common endocrine diseases in dogs, resulting in a low concentration of thyroid hormone, which may occur due to thyroid gland destruction, decreased stimulation by the thyroid stimulation hormone (TSH) from the pituitary gland, or interfering with the cascade of thyroid synthesis and regulation. Primary hypothyroidism can be classified as either congenital or acquired. Congenital hypothyroidism is characterized by a decrease in the production and secretion of TSH, which is rare in dogs (da Silva Meirelles *et al.*, 2017). Ninety-five percent of primary hypothyroidism cases involved the progressive loss of thyroid tissue presented by immune-mediated destruction (canine lymphocytic thyroiditis; CLT) and idiopathic atrophy in middle-aged dogs by equal percentages (Scott-Moncrieff *et al.*, 2015; Kemppainen and Behrend, 2001). CLT is divided into 4 stages according to the degree of lymphocyte cell destruction in the thyroid gland (Graham *et al.*, 2007). CLT is a complex disease caused by unclear predisposing genetic and environmental risk factors (Happ and Medicine, 1995).

Thyroid hormones have a variety of physiological effects. When the thyroid hormone level is below normal, it can have profound clinical effects on body systems, including reducing the metabolic rate and showing clinical signs of inactivity, lethargy, and weight gain. Dermatological signs occur in 60–80% of hypothyroid dogs (Scott-Moncrieff, 2012). Other clinical signs result from functional changes in the neuromuscular, cardiovascular, reproductive, and gastrointestinal systems (Scott-Moncrieff *et al.*, 2015). Approximately 30% of affected dogs were reported to have hematological abnormalities associated with nonregenerative anemia (Panciera, 2001). In addition, hypercholesterolemia was reported in 75% of cases (van Hoek and Daminet, 2009) despite decreasing thyroid synthesis in this condition, the diminished thyroid hormone degradation rate affected more than synthesis, with the evidence being an accumulation of plasma lipids. The glomerular filtration rate (GFR) may decrease in the kidneys and serum creatinine may increase via pre-renal and renal effects in hypothyroidism (van Hoek and Daminet, 2009). Hypothyroidism progresses slowly as a disease; therefore, the clinical symptoms are difficult to recognize until the end stage of the disease. Delays in diagnosis will not only underestimate the incidence rate and prevalence but can also lead to increased risk of morbidity and mortality related to the multiorgan system. A human meta-analysis reported that heart failure, coronary artery disease episodes, and mortality from coronary heart disease appear to be more common in patients with subclinical hypothyroidism (Biondi *et al.*, 2019). Some of the subclinical hypothyroid patients were thyroid antibody-positive (approximately 2.1–3.8%), with 0.3% thyroid antibody-negative (Samuels, 1998). To our knowledge, there has been no published veterinary medical report of grading in subclinical hypothyroidism. If there are clinical characteristics predicting the possibility of this disease, then dogs with subclinical hypothyroidism could be diagnosed.

Canine breed populations may influence geographical variation; based on the measurement of serum thyroglobulin autoantibody, many breeds have increased lymphocytic thyroiditis incidence and have a hereditary predisposition, including English Setter, Old English Sheepdog, Boxer, Giant Schnauzer, American Pit Bull Terrier, and Beagle (Milne and Hayes Jr, 1981; Benjamin *et al.*, 1996). There is a lack of available information on canine hypothyroidism in Thailand. The first aim of this retrospective study was to evaluate the demographic data that influence the prevalence of hypothyroidism. The second aim was to investigate the risk factors for analyzing clinical signs and clinicopathological data in hypothyroid dogs to suggest which variables could be prognostic factors.

Materials and Methods

The study was approved by the Kasetsart University Institutional Animal Care and Use Committee (ACKU64-VET-076).

Study design: The study was designed as a retrospective cross-sectional study. The medical records of dogs in which a concurrent measurement of serum canine total thyroxine (cTT4) and serum canine thyroid-stimulating hormone (cTSH) at the Kasetsart University Teaching Hospital, Bangkok, Thailand, from January 2019 to September 2021 were collected. Information collected from medical records included signalment, body condition score (BCS; 9-point scale), history and clinical signs (dermatological abnormalities, lethargy, exercise intolerance, neurological abnormalities). The dogs with BCS equal or greater than 7 were identified as obese dogs. Inclusion criteria identified 2 groups: non-hypothyroid dogs whose clinical signs were consistent with hypothyroid disease but who had a normal thyroid level (normal level of serum cTT4 [1.0 –4.0 µg/dL] and serum cTSH [0 –0.5 ng/mL]) and secondly, hypothyroid dogs (low levels of cTT4 and high levels of cTSH) (Kemppainen and Behrend, 2001). Dogs were excluded if there were any of congenital hypothyroidism (low level of serum cTT4 and high levels of cTSH under one year of age), non-thyroidal illness (NTI), thyroidectomy, received medication affecting thyroid hormone, or received thyroid hormone replacement treatment within the previous week. Phenobarbital administration in epilepsy dogs of any group may cause decreased cTT4, but cTSH concentrations usually not elevated than in the upper limit of the reference range was considered as an inclusion criterion (Gieger *et al.*, 2000). Based on the retrospective study, dogs with concurrent endocrine disorders were not excluded.

Clinicopathological evaluation: All blood samples were collected from routine venipuncture. Complete blood cell counts were measured using an automated hematology analyzer (The Sysmex XN-1000®; Sysmex Corp.; Kobe, Japan) and serum blood chemistry (blood urea nitrogen (BUN), creatinine (CR), alanine aminotransferase (ALT), alkaline phosphatase (ALP),

cholesterol (CHO), triglyceride (TG), albumin (ALB)) were measured using an automated chemistry analyzer (ILab Tarsus; Instrumentation Laboratory; Milan, Italy). The serum cTT4 and serum cTSH were determined using commercially available chemiluminescent enzyme immunometric assays (Immulite 1000; Siemens Medical Solutions Diagnostics; Los Angeles, CA, USA).

Statistical analysis: Data were evaluated for normality based on the Shapiro-Wilk test. Normally distributed data were reported as mean ± SD, and nonnormally distributed data were reported as median and range. The descriptive categorical variables were reported as counts (n) and percentages (gender, breed, obesity, and others). The demographic breed distribution of hypothyroid dogs was compared using Pearson's Chi-squared test or Fisher's exact test to calculate the odds ratio (OR) and the 95% confidence interval (95% CI). The continuous variable between groups was compared using an unpaired Student's *t*-test or a Mann-Whitney *U* test.

The dichotomous variables were breed (breed versus mixed breed), obesity (BCS ≥ 7 versus BCS < 7), skin lesion include alopecia, dry skin, poor hair coat quality, seborrhea, superficial pyoderma, rat tail, epidermal collarette, and lichenification (skin lesion versus not found skin lesion), and neurological sign include peripheral neuropathy, cranial nerve dysfunction, and cerebral dysfunction (neurological sign versus not found neurological sign). Risk factor analysis was based on: bradycardia (heart rate ≤ 60 beats per minute versus heart rate > 60 beats per minute), anemia (packed cell volume; PCV < 35% versus PCV ≥ 35%), azotemia based on International Renal Interest Society (IRIS ≥ stage 2 versus IRIS < stage 2), hypercholesterolemia (CHO ≥ 335 mg/dL versus < 335 mg/dL), hypertriglyceridemia (TG ≥ 120 mg/dL versus < 120 mg/dL), hypoalbuminemia (ALB < 2.3 g/dL versus ≥ 2.3 g/dL) and polychotomous variables were ALT and ALP (normal, 2–3 reference interval, > 3 reference interval) (Paula 2011; Xenoulis and Steiner 2010). Based on univariate analysis, age and gender were identified as statistically significant of confounder (P < 0.1). The findings that were statistically significant using univariate analysis were entered into multivariate analysis (backward elimination) to determine their independent values associated with canine hypothyroidism. Effect-adjusted measures of association were reported as OR with a 95% CI. A value of P < 0.05 was considered significant, performed using commercial statistical software packages for all statistical analyses (NCSS11, NCSS LLC., USA).

Results

Population demographic data: In total, 256 dogs (77 hypothyroid, 179 non-hypothyroid groups) met the inclusion criteria (Figure 1). The period prevalence of hypothyroidism in the suspected hypothyroid population and the whole hospital population were 11.5% and 0.23% respectively.

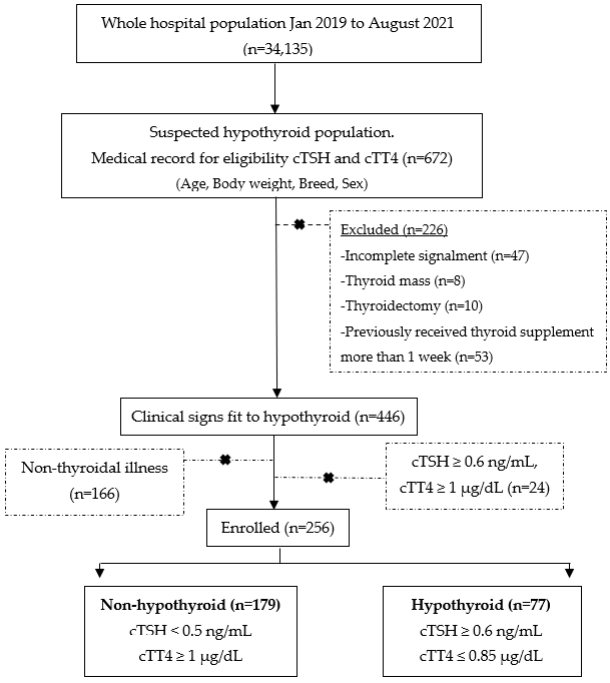

Figure 1 Flow diagram showing dog enrollment for this study, where dotted boxes refer to excluded dogs. Non-thyroidal illness refers to low levels of canine total thyroxine, cTT4 ≥0.85 µg/dL) and normal levels of canine thyroid-stimulating hormone, cTSH (0-0.5 ng/mL). All cTT4 levels below the reference range and normal levels of cTSH were classified as NTI. In the excluded group, the thyroid mass consisted of dogs that underwent thyroidectomy and histopathologic adenoma or carcinoma. Based on limitation of measuring recombinant human TSH stimulation tests, the confirmation of true hypothyroid dogs in that condition (low cTT4 and normal TSH) could not be distinguished from NTI. The high cTSH levels with normal levels of cTT4 are either an early stage of primary hypothyroidism or hyperfunction of TSH-releasing thyrotrophs. We could not perform TgAA evaluation or a triiodothyronine (TT3) suppression test, so this condition also was excluded.

There were 17 breeds included in the hypothyroid group, as shown in **Figure 2**: The highest breed was Thai Bangkaew dogs (31.2%, 24/77), Crossbreed dogs (23.4%, 18/77), Pomeranian (10.4%, 8/77), Beagle (7.8%, 6/77), and Chihuahua (6.5%, 5/77) respectively. The hypothyroid group consisted of 52% male (40/77) and 48% female (37/77).

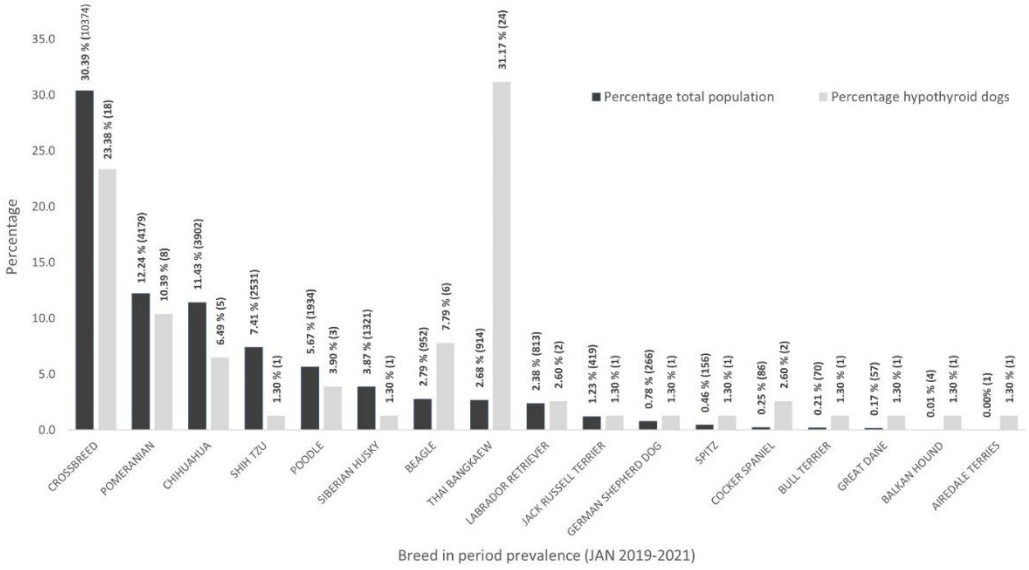

Figure 2 Breed distribution of dogs with primary hypothyroidism compared to matching breed population in Kasetsart University Veterinary Teaching Hospital, Bangkok, Thailand.

Note: The total population of dogs recorded from January 2019 to September 2021 was 34,135 and the total number of hypothyroid dogs was 77. The black bar graph depicts the percentage of each breed population, with the gray bar graph depicting the percentage of breed in the hypothyroid group in the same period. The numbers in brackets show the absolute number in each group. A value of 0.00 represents a percentage less than 0.01.

The most common breed in non-hypothyroid group were Crossbreed dogs (24%, 43/179), Pomeranian (13.4%, 24/179), Shih Tzu (11.2%, 20/179), Thai Bangkaew (9.5%, 17/179), and Golden Retriever (7.3%, 13/179) respectively. The breed information in the hypothyroid and non-hypothyroid groups that match is presented in Figure 2. The non-hypothyroid group consisted of 49.7% male (89/179) and 50.3% female (90/179) dogs. The median age at the time of diagnosis in the hypothyroid dogs (8 years, range 3–16.1 years) was significantly lower than in the non-hypothyroid dogs (9.1 years, range 2–16 years; P = 0.01). There were no significant differences in gender and body weight between the hypothyroid and non-hypothyroid groups (Table 1)

Table 1 Descriptive variables for 256 dogs classified as hypothyroid or non-hypothyroid.

Variable	Hypothyroid	Non-hypothyroid	*P*-value
Number of dogs	77	179	N/A
Sex (male/female)	40/37	90/91	0.74
Age (year.month)	8[3–16.1]	9.1[2–16]	0.01
Body weight (kg)	20.9[2.7–51.2]	14.92[1.5–55.7]	0.16
Obesity (yes/no)	36/14	88/57	0.14

Note: Values indicate statistical significance at $P < 0.05$
Obesity, body condition score ≥ 7 (range of scores:1–9).

Figure 2 illustrates the period prevalence among 34,135 dogs at Kasetsart University. Among the entire hospital population, the Thai Bangkaew breed had a significantly higher risk of 17.45 times [OR 17.45, 95% CI, 10.16–28.76; $P <$ 0.001] while the Beagle breed had a 2.93-fold higher risk [OR 2.93, 95% CI, 1.57–7.19; P = 0.02]. However, within the 672 dogs matching clinical signs of hypothyroidism in our study, the Thai Bangkaew breed had a 3.63-fold higher risk of developing hypothyroidism [OR 3.63, 95% CI, 2.15–6.25; $P <$ 0.001], as indicated in Table 2.

Table 2 Breed distribution for dogs with primary hypothyroidism compared to matching breed population in the Kasetsart University Veterinary Teaching Hospital, Bangkok, Thailand.

Breed	Hypothyroid dogs n (%)	Hypothyroid in matching breed population during study period n (%)[1]	Hypothyroid in all suspected population n (%)[2]
Crossbreed	18 (23.38%)	63 (28.57%)	150 (12%)
Pomeranian	8 (10.39%)	36 (22.22%)	107 (7.48%)
Chihuahua	5 (6.49%)	29 (17.24%)[a]	35 (14.29%)[a]
Poodle	3 (3.90%)	28 (10.71%)[a]	32 (9.38%)[a]
Beagle	6 (7.79%)	20 (30%)*	44 (13.64%)
Thai Bangkaew	24 (31.17%)	44 (54.54%)**	90 (26.67%)**

Note: There were 77 dogs in the hypothyroid group and 179 non-hypothyroid dogs. There was no match between groups (e.g., rare breeds) for Airedale Terrier, Balkan Hound, and Great Dane in the hypothyroid group and for Maltese, Miniature Pinscher, Pug, and Yorkshire Terrier in the non- hypothyroid dogs. Coefficients and confidence intervals are based on logistic regression.

[a]Asymptotic tests were used except when there were <5 observations in a cell and then Fisher's exact test was used

[1] Number of dogs visiting the hospital from January 2019 to August 2021 (n=34,135)

[2]Number of dogs suspected hypothyroid from January 2019 to August 2021 (n=672)

* Values indicate statistical significance at $P < 0.05$; ** Values indicate statistical significance at $P < 0.001$.

Association of hypothyroidism between pure breed and crossbreed: The risk for diagnosis of canine hypothyroidism was higher in Thai Bangkaew (Table 3); which showed increased odds compared with crossbreeds as a reference (OR 3.37, [95% CI, 1.47–7.73; $P < 0.001$]), while the protective breed for hypothyroidism was Shih Tzu (OR 0.12, [95% CI, 0.01–0.96; $P < 0.05$]).

Table 3 Association of purebred data in primary hypothyroidism compared to crossbreed (mixed breed) at Kasetsart University Veterinary Teaching Hospital, Bangkok, Thailand.

Breed	Disease	No disease	OR	95% CI of OR
Crossbreed	18	43	Ref	Ref
Beagle	6	9	1.59	0.49-5.13
Chihuahua	5	9	1.33	0.39-4.51
Cocker Spaniel	2	1	4.78	0.41-56.07
German Shepherd	1	1	2.39	0.14-40.31
Labrador Retriever	2	7	0.68	0.13-3.61
Pomeranian	8	24	0.80	0.30-2.10
Poodle	3	6	1.19	0.27-5.31
Shih Tzu	2	20	0.12*	0.01-0.96
Siberian Husky	1	11	0.22	0.03-1.81
Spitz	1	1	2.39	0.14-40.31
Thai Bangkaew	24	17	3.37*	1.47-7.73

Note: Coefficients and confidence intervals are based on logistic regression.

* Values indicate statistical significance at $P < 0.05$.

Clinical signs and laboratory abnormalities: Dermatological abnormalities were the most common clinical symptoms of hypothyroid dogs (59.2%; 45/76), and they were substantially more prevalent in hypothyroid dogs than in non-hypothyroid dogs (27%; 48/178) (OR 4.0, [95% CI, 2.18–7.30; $P = 0.001$]). These skin problems included alopecia, rat tail, epidermal collarette, lichenification, coarse hair, and superficial pyoderma. Neurological signs were more common in hypothyroid dogs (15.8%; 12/76) than in non-hypothyroid dogs (12.8%; 23/179), but these differences were not significant [95% CI, 0.55–2.89; $P = 0.65$]. Neurological signs included vestibular signs, peripheral neuropathy, hemiparesis, ataxia, and seizure. Other clinical signs in hypothyroid dogs were inactivity (10.5%; 8/76), bradycardia (8.2%; 4/49), and weight gain (1.3%; 1/76)

The clinicopathological laboratory results are presented in Table 4. The median hematocrit value in hypothyroid dogs (36.3%, range 20.9–62.5%) was significantly lower than in non-hypothyroid dogs (43%, range 30.8–57.2%; $P < 0.001$), with mild anemia in hypothyroid dogs detected in 39.5% (30/76) of cases, while non-hypothyroid dogs were detected in 9.6% (17/178) of cases.

Table 4 Clinicopathological findings from 256 dogs, classified as hypothyroid or non-hypothyroid.

Variable	Hypothyroid	Non-hypothyroid	*P-value*
Number of dogs	77	179	N/A
Complete blood cell count (CBC)			
PCV (%)	36.3[20.9–62.5]	43[30.8–57.2]	<0.001
WBC (U/L)	11.6[4.61–41.79]	10.78[4.09–34.02]	0.48
Platelet (x10³/uL)	309[20–787]	293[81–772]	0.52
Blood chemistry			
Serum cTT4 (µg/dL)	0.49[0.26–0.82]	1.61[1.01–3.69]	<0.001
Serum cTSH (ng/mL)	1.38[0.61–10.8]	0.17[0.03–0.4]	<0.001
Serum TG (mg/dL)	179.5[54–1,254]	132.5[19–1,626]	0.06
Serum CHO (mg/dL)	367[159–852]	279.5[121–629]	<0.001
Serum BUN (mg/dL)	18[3–197]	17[3–51]	0.77
Serum CR (mg/dL)	1.25[0.48–5.98]	0.94[0.4–2.19]	<0.001
Serum ALT (U/L)	52.5[12–667]	51[4–823]	0.91
Serum ALP (U/L)	90[10–11,697]	152[12–19,324]	0.41
Serum ALB (g/dL)	2.9[1.9–4]	3.2[1.4–4.4]	<0.001

Note: P-value in each row, indicate significant differences between groups based on the Man- Whitney U test. The level of significance was $P < 0.05$.

PCV, packed cell volume; WBC, white blood cell count; cTT4, canine total thyroxine; cTSH, canine thyroid-stimulating hormone; TG, triglyceride; CHO, cholesterol; BUN, blood urea nitrogen; CR, creatinine; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ALB, albumin.

The median serum creatinine levels in hypothyroid dogs (1.25 mg/dL, range 0.48–5.98 mg/dL) were significantly higher than in non-hypothyroid dogs (0.94 mg/dL, range 0.4–2.19 mg/dL; $P < 0.001$), though within the reference intervals. Azotemia occurred in 34.2% (26/76) of hypothyroid dogs and 10.7% (19/178) of non-hypothyroid dogs. Hypothyroid dogs had significantly higher median serum cholesterol levels (367 mg/dL, range 159–852 mg/dL) compared to non-hypothyroid dogs (279.5 mg/dL, range 121–629 mg/dL; $P < 0.001$). Hypercholesterolemia was observed in 56.8% (21/37) of hypothyroid cases and 19.4% (21/108) of non-hypothyroid cases. Hypertriglyceridemia was more common in hypothyroid dogs (72.2%, 26/36) compared to non-hypothyroid dogs (53.7%, 58/108), with no significant differences between the groups. The median serum albumin levels in hypothyroid dogs (2.9 g/dL, range 1.9–4.0 g/dL) were significantly lower than in non-hypothyroid dogs (3.2 g/dL, range 1.4–4.4 g/dL), but still within the reference intervals for both groups.

***Identification of risk factors for hypothyroidism*:** Initially 13 variables were investigated for their potential association with perceived susceptibility of hypothyroidism in dogs based on a univariable logistic regression model (Table 5). Among these variables, for the Thai Bangkaew breed (OR 4.32, [95% CI, 2.15–8.64]), the presence of skin lesions (OR 3.93, [95% CI, 2.24–6.91]), bradycardia (OR 5.07, [95% CI, 0.90–28.64]), anemia (OR 4.32, [95% CI, 2.24–8.32]), azotemia (OR 6.18, [95% CI, 3.13–12.18]), hypercholesterolemia (OR 5.44, [95% CI, 2.43–12.18]), hypertriglyceridemia (OR 2.24, [95% CI, 0.99–5.10]), and hypoalbuminemia (OR 3.86, [95% CI, 1.28–11.61]) were associated with a significant risk of canine hypothyroidism ($P < 0.1$) and were further selected for multivariable analysis for determine the relative influences of these variables on hypothyroid dogs .

Table 5 Univariable association between variables and disease outcome in dogs with primary hypothyroidism.

Variable		Disease Yes	No	Regression coefficient(β)	OR	95% CI
Categorical variables						
Age (years)	1-4	14	20	0.16	1.17	1.02–1.35
	5-8	31	49	0.22	1.25	0.84–1.87
	>8	32	110	-0.55	0.58	0.39–0.84
Thai Bangkaew breed[a]	No	53	17	-0.27	0.76	0.65–0.89
	Yes	24	162	1.46	4.32	2.15–8.64
Obesity	No	14	57	-0.35	0.70	0.43–1.14
	Yes	36	86	0.53	1.70	0.84–3.44
Skin lesion[a]	No	31	130	-0.58	0.56	0.42–0.74
	Yes	45	48	1.37	3.93	2.24–6.91
Neurological lesion	No	64	156	-0.03	0.97	0.86–1.08
	Yes	12	23	0.24	1.27	0.60–2.71
Bradycardia[a]	No	45	114	-0.07	0.93	0.86–1.02
	Yes	4	2	1.62	5.07	0.90–28.64
Anemia[a]	No	46	161	-0.28	0.76	0.66–0.87
	Yes	30	17	1.46	4.32	2.24–8.32
Azotemia[a]	No	50	159	-0.40	0.67	0.55–0.81
	Yes	26	19	1.82	6.18	3.13–12.18
Hypercholesterole-mia[a]	No	16	87	-0.62	0.54	0.37–0.79
	Yes	21	21	1.69	5.44	2.43–12.18
Hypertriglyceride-mia[a]	No	10	50	-0.51	0.60	0.34–1.06
	Yes	26	58	0.81	2.24	0.99–5.10
Elevated ALT	Normal	61	140	0.13	1.14	0.70–1.84
	2-3 RI	12	35	-0.34	0.72	0.36–1.39
	>3 RI	3	4	0.44	1.56	0.56–4.34
Elevated ALK	Normal	38	66	-0.17	0.84	0.71–1.00
	2-3 RI	24	45	0.42	1.53	0.99–2.36
	>3	8	58	-0.93	0.40	0.23–0.68
Hypoalbuminemia[a]	No	56	162	-0.09	0.91	0.82–1.00
	Yes	8	6	1.35	3.86	1.28–11.61

Note: Coefficients and confidence intervals based on logistic regression.
[a] Included in the final multivariable model.
ALT, alanine aminotransferase; ALP, alkaline phosphatase.

The final model identified the following independent risk factors for canine hypothyroidism: Thai Bangkaew breed, presence of skin lesion, anemia, hypercholesterolemia, and hypertriglyceridemia (Table 6). Thai Bangkaew dogs with a skin lesion, anemia, hypercholesterolemia, and hypertriglyceridemia had a significantly increased risk of 4,272 times for canine hypothyroidism compared to the non-Thai Bangkaew breed without skin lesion and with normal cholesterol, and triglyceride levels.

Table 6 Association of multivariable outcomes in canine primary hypothyroidism.

Variable	Regression Coefficient (β)	Standard Error	*P-value*	Exp (β) OR	95% of CI
Thai Bangkaew breed	2.10	0.67	0.002	8.21	2.21–30.45
Skin lesion	1.98	0.53	<0.001	7.24	2.54–20.63
Hypercholesterolemia	1.55	0.53	0.004	4.70	1.65–13.38
Hypertriglyceridemia	1.17	0.59	0.047	3.21	1.01–10.16
Anemia	1.56	0.67	0.019	4.78	1.28–17.83

Note: The level of significance was $P < 0.05$.

The laboratory results identified that among Thai Bangkaew dogs with skin lesions, those with hypercholesterolemia and hypertriglyceridemia had a remarkably higher risk of 897 times for canine hypothyroidism compared to non-Thai Bangkaew breed dogs that were normocholesterolemic and normotriglyceridemic without skin lesions. Thai Bangkaew dogs without skin lesions but the presence of anemia, hypercholesterolemia, and hypertriglyceridemia had 589 times higher risk of canine hypothyroidism compared to non-Thai Bangkaew breeds without skin lesions and with normal cholesterol and triglyceride levels. In routine physical examination, Thai Bangkaew dogs with skin lesions had a 59 times greater risk of canine hypothyroidism compared to non-Thai Bangkaew breed dogs without skin lesions.

Furthermore, when adjusted, skin lesions, anemia, hypercholesterolemia, and hypertriglyceridemia were 8.21 times greater in Thai Bangkaew dogs than non-Thai Bangkaew breed dogs in association with hypothyroidism for the same conditions.

Discussion

We found 5 independent risks for canine hypothyroidism in this study, including Thai Bangkaew dogs, skin lesions, anemia, hypercholesterolemia, and hypertriglyceridemia. We proposed that Thai Bangkaew is the breed to predict

the risk for hypothyroid disease based on geographical variation.

According to this study, the period prevalence of hypothyroidism in dogs was 0.23%, consistent with other research (0.2–0.8%) (Panciera, 1994; Dixon *et al*., 1999). The age of the first hypothyroid diagnosis may predict the typed rate of progression. The peak prevalence of detection of anti-thyroglobulin antibodies (TgAA) is 2–4 years of age, while TgAA negatives are usually found for the onset of hypothyroidism between 5 and 8 years of age (Graham *et al.,* 2007), consistent with other study (Kour *et al*., 2020). Our finding was that the median age of hypothyroid dogs was 8 years, similar to other studies that defined the late stage of the disease. We assumed that idiopathic thyroid atrophy was likely the source of the disease, based on the median age at diagnosis. To distinguish the lymphocytic thyroiditis and idiopathic atrophy based on histological finding is very invasive in patients (Conaway *et al.,* 1985). Early diagnosis using the TgAA will improve the management of this disease. Further studies may use combined TgAA testing with cTT4 and cTSH in the predisposing breed or in dogs with risk factors to forecast the possibility of developing lymphocytic thyroiditis.

Geographical variation and breed popularity may affect the breed incidence reports in each area. The breeds with the most reported incidence of hypothyroidism in USA data were the Golden Retriever and Doberman Pinscher (Milne and Hayes Jr, 1981), while in Swedish data they were the Giant Schnauzer and Hovawart (Ferm *et al*., 2009). The strong breed predispositions for diagnosis of hypothyroidism in the UK were the Doberman Pinscher, Tibetan Terrier, and Boxer (O'Neill *et al*., 2022). Asian data reported the Labrador retriever as the highest prevalence breed in India (Kour *et al*., 2020). Our study reported the highest risk for hypothyroidism was for the Thai Bangkaew (31.2%). To our knowledge, this was the first report of the increased prevalence of the Thai Bangkaew breed in dogs with hypothyroidism. The Thai Bangkaew is the second Thai breed approved by The Belgium-based Federation Cynologique Internationale (FCI) and registered in Group 5 of the Spitz and primitive dogs in 2022, after the Thai Ridgeback in 2003. Thai Bangkaew breed traces its ancestry back to a cross between a native black and white female dog and the now-extinct wild wolves which are believed to have originated as a species in Phitsanulok province, Thailand (Phavaphutanon and Laopiem, 2011). Furthermore, purebred dogs are particularly prone to develop autoimmune diseases; our results showed the Thai Bangkaew breed had a higher frequency of hypothyroid dogs compared with Crossbreed (OR = 3.37), consistent with the results reported from primary veterinary care in UK and US referrals, where purebred dogs have 1.49 and 1.56 times, respectively, odds of hypothyroidism compared to Crossbreed dogs (O'Neill *et al*., 2022; Bellumori *et al*., 2013). The proportion of TgAA-positive has never been reported in Thai Bangkaew dogs based on our knowledge. To understand the genetic risk factors for the development of canine lymphocytic thyroiditis, further studies should be undertaken on dog leukocyte antigen-performed (DLA) class II genes together with TgAA to identify the association predisposing genetic risk factor hypothyroidism in Thai Bangkaew dogs.

Beagle is one of the breed risk factors for hypothyroidism (Benjamin *et al.,* 1996; O'Neill *et al*., 2022), for an association has been reported with TgAA-positive (Graham *et al*., 2007; Ferguson, 2007). However, the current study identified increased odds of risk hypothyroidism only with matching breed population during study period (OR = 2.93). This observation serves as a reminder to focus on a Beagle breed investigation of genetic loci associated with hypothyroidism in further study.

The protective breed in the current study was the Shih Tzu, similar to those reported in the UK (O'Neill *et al*., 2022). Other protective breeds in the UK were the Pug, Yorkshire Terrier, and Jack Russell Terrier, while a US investigation into purebred predisposition to hypothyroidism showed no identified Terrier and Toy breed groupings (Oberbauer *et al*., 2015). The genetic risk factor gene report of DLA class II in the Giant Schnauzer, Dobermann, and Labrador Retriever was the DLA-DRB1*01201/DQA1*00101/DQB1*00201 haplotype, whereas DLA-DRB1*01301/DQA1*00301/DQB1*00501 haplotype was the protective gene for developing the disease in the Giant Schnauzer (Wilbe *et al*., 2010). Although the theories regarding evidence in small and toy breeds are unclear and evidence is limited, future research should explore the genetic variety of DLA in high-risk and protective breeds compared to hypothyroid and non-hypothyroid dogs.

Our study demonstrated that the levels of PCV and ALB significantly decreased in hypothyroid dogs compared to non-hypothyroid dogs. In contrast, CR levels were significantly higher in hypothyroid dogs than in non-hypothyroid dogs. However, the median levels of PCV, ALB, and CR were still within the reference level in our study. In the previous study, the median PCV values remained within the reference range, and a similar percentage range 33-40% showed signs of anemia (Corsini *et al*., 2021; Guglielmini *et al*., 2019; Di Paola *et al*., 2021), which is consistent with the current study's findings of a 39.5% prevalence. In the current study, the creatinine levels were above the reference level in one-third of the hypothyroid dogs, which agreed with other studies (Dixon *et al*., 1999; Gommeren *et al*., 2009) but was in contrast to another study (Panciera and Lefebvre, 2009). Another study using serum symmetric dimethylarginine (SDMA) to assess the accuracy of kidney function in hypothyroid dogs not found statistically significant differences (Di Paola *et al*., 2021).

Albumin plays a crucial role in stabilizing thyroid hormone in circulation and influences renal function via the metabolism and elimination of thyroid hormone (Foley, 2008). In human patients, the association has been demonstrated of nephrotic syndrome (NS) with thyroid hormone dysfunction (Li *et al*., 2019). To our knowledge, the current study was the first published report of significantly decreased ALB levels in hypothyroid dogs, although the median level of albumin in this study was still within the normal limits. Further study is encouraged to investigate the association of renal disease and

hypothyroidism in dogs with gradually albumin reduction.

The thyroid hormone has direct regulation of both lipid synthesis and metabolism. In a human report, it was expected there would be crosstalk between thyroid hormone receptors (THRs) and liver X receptors (LXRs) but this could not still in elucidation on molecular mechanisms (van Heyningen and Glaysher, 2012). Furthermore, triiodothyronine on the THRβ1 isoform (THR β1) may promote cholesterol excretion by stimulating reverse cholesterol transport and transintestinal cholesterol excretion (Duntas and Brenta, 2012; Pramfalk *et al.,* 2011). We found that the median levels of CHO were significantly higher than the reference level in hypothyroid dogs compared to non-hypothyroid dogs. In addition, hypercholesterolemia was reported at about 56.8% in our study, which was a lower frequency than the 67–91% reported in other studies (Mooney, 2011; Guglielmini *et al.,* 2019; Di Paola *et al.,* 2021), probably because of the nature of our retrospective design. In our study, hypertriglyceridemia was 72.2%, which was comparable to another study (75%) (Corsini *et al.,* 2021).

Hypothyroid deficiency is a slowly progressive disease, with the clinical signs only clearly shown when the disease has become severe. Furthermore, nonspecific clinicopathological abnormalities may conflict with other geriatric conditions. Therefore, the current study could provide the most important risk factors to seeking a proper screening diagnosis of thyroid disease. The Thai Bangkaew breed with anemia, hypercholesterolemia, hypertriglyceridemia, and skin lesions had 4,272 times greater diagnosed hypothyroidism. This finding highlights a potential factor for generating clinical scores and grading systems in further study by prospective study design for accurate prediction in suspected hypothyroid dogs. Adding lipid profile monitoring in routine health programs would be advisable in Thai Bangkaew dogs, which suggests a strong breed predisposition in our study (OR = 8.21).

A limitation in our study was that it was based on a retrospective study design. Not all dogs had complete data records, and because of low case numbers, we could not determine some statistically significant variables in other studies, such as bradycardia. Interobserver agreement between clinicians was not able to be controlled. It is possible that our research underestimated the hypothyroid group because of the lack of recombinant human TSH stimulation tests performed on NTI group. About 20–40% of hypothyroid dogs have a normal TSH concentration (Dixon *et al.,* 1999; Shiel *et al.,* 2010); however, we assumed that significant risk factors in our study could provide a potential benefit for veterinary practitioners and further prospective study design.

This study indicated the potential association of risk factors, including anemia, hypercholesterolemia, hypertriglyceridemia, and skin lesions, suggesting a strong diagnostic characteristic for canine hypothyroidism. Evaluation of serum cholesterol and triglyceride in health programs for Thai Bangkaew dogs would be advisable in screening before investigating the thyroid profile, indicating that it is a common genetic risk factor in various breeds.

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