

EUTHYROID SICK SYNDROME IN CANINE BABESIOSIS CAUSED BY *BABESIA CANIS*

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Abstract

The aims of this study were estimation of thyroxin status in dogs infected with *B. canis*, and determination of the association between azotaemia and concentrations of total thyroxin (TT4) and free thyroxin (FT4) in canine babesiosis. Concentrations of TT4 and FT4 were determined using an immunoassay in 23 dogs infected with *B. canis* (nine azotaemic dogs and 14 non-azotaemic dogs). Mann-Whitney *U* test was used to compare the concentrations of TT4 and FT4 in groups of azotaemic and non-azotaemic dogs. Correlations between azotaemic parameters (*i.e.* serum urea and creatinine) and TT4 and FT4 concentrations were calculated. The obtained results showed high prevalence of dogs infected with *B. canis* showing decreased TT4 and FT4 concentrations. No difference between concentrations of TT4 and FT4 in azotaemic and non-azotaemic dogs was demonstrated, but statistically significant correlations between the level of TT4 and FT4 and serum levels of urea and creatinine were shown.

Key words: dogs, *Babesia canis*, canine babesiosis, azotaemia, euthyroid sick syndrome, nonthyroidal illness syndrome.

Babesia canis (formerly: *Babesia canis canis*) is the only species of the genus *Babesia*, which infects dogs in Poland (1, 13, 15). The infection leads to the development of canine babesiosis, which is a severe systemic disease. Kidney and liver failure, heart damage, and injury of other organs were observed in affected dogs (4, 8, 11). Such severe systemic diseases cause euthyroid sick syndrome (also known as nonthyroidal illness syndrome), which was also observed in canine babesiosis caused by infection with *B. rossi* in South Africa (6, 9). This syndrome results from increased production of pro-inflammatory cytokines such as TNF α and IL-6, which leads to inhibition of the hypothalamic-pituitary-thyroid axis function. This results in a decrease in the production and secretion of thyroid hormones (thyroxin and triiodothyronine), leading to a decrease in serum concentration of thyroid hormones (3). The degree of the decrease in serum thyroxin concentration in canine babesiosis caused by *B. rossi* was connected with the severity of the disease and mortality in affected dogs (9). Decreased serum concentration of thyroid hormones is also observed in the course of hypothyroidism, which in dogs results mainly from lymphocytic thyroiditis and idiopathic thyroid atrophy (12).

Thyroid hormones affect carbohydrate and lipid metabolism by accelerating and facilitating biochemical transformations and increasing oxygen consumption in

tissues. Moreover, these hormones affect the heart causing inotropic and chronotropic effects, which results in an increase in blood pressure (7). In hypothyroidism, a decreased cardiac output is associated with a decrease in renal blood flow and glomerular filtration rate. In hypothyroid humans, glomerular filtration rate may be reduced up to 40% and is associated with an increase in serum creatinine concentration (12).

The authors of this work hypothesised that a decreased concentration of thyroxin in dogs infected with *B. canis* may be associated with azotaemia resulting from a decreased renal blood flow. In this study thyroxin concentration status in *B. canis* infected dogs and association between azotaemia and serum thyroxin concentration in canine babesiosis were investigated.

Material and Methods

Serum and blood samples from 23 dogs infected with *B. canis* were collected. The infection was detected by blood examination, using blood smears stained with Giemsa, and confirmed by the PCR method described in the previous papers (10, 15). All dogs were presented to the Center of Small Animal Health Clinic Multiwet (Warsaw) with clinical signs of canine babesiosis, such as: fever, apathy, anorexia, vomiting, diarrhoea, pale

mucous membranes, and dehydration. Samples of the blood and serum were collected before treatment and during 1-4 d of disease duration. Excluded criteria were as follows: earlier diagnosis of hypothyroidism or history of clinical signs suggesting hypothyroidism, any drug therapy in the preceding 4 weeks (including dogs misdiagnosed with babesiosis), history of hormonal therapy in the preceding 6 months, known concurrent disease or infection, history of travelling abroad in the preceding one year, and inability to clearly identify azotaemia (*i.e.* increased concentration of serum urea and creatinine concentration within reference intervals in the same sample).

Ethylenediamine tetraacetic acid (EDTA) was used as an anticoagulant for the examination of the whole blood (including PCR examination). Serum was obtained by the centrifugation of blood samples collected in tubes without an anticoagulant. Serum concentrations of urea, creatinine, and cholesterol were determined by a clinical chemistry analyser (XL 640, Erba Mannheim, Germany). Total thyroxine (TT4) and free thyroxine (FT4) concentrations were determined by an immunoassay analyser DPC IMMULITE ONE (Siemens Medical Solutions Diagnostics) using diagnostic kits, namely, IMMULITE Canine Total T4 and IMMULITE Free T4 (Siemens Healthcare Diagnostics).

The obtained results allowed dividing the serum samples into two groups: group A (azotaemic dogs), group B (non-azotaemic dogs). The results were analysed using the Statistica 8.0 programme. Mann-Whitney *U* test was used to compare the concentrations of TT4, FT4, and cholesterol in both groups. Correlations between azotaemic parameters (*i.e.* serum

urea and creatinine) and TT4 and FT4 concentrations were calculated. The value of $P < 0.05$ was considered significant.

Results

Decreased TT4 concentration was detected in 21 out of 23 dogs (91.3%; 95% confidence interval (CI) - 78.8% to 100%). Decreased concentration of FT4 was detected in 19 out of 23 dogs (82.61%; 95% CI - 65.85% to 99.37%). Cholesterol concentration was within reference interval in all 23 dogs. Medians and mean concentrations of TT4, FT4, and cholesterol in 23 infected dogs are presented in Table 1.

Azotaemia was detected in nine out of 23 dogs (39.13%; 95% CI - 17.55% to 60.71%, Group A). The rest of the dogs (60.87%; 95% CI - 39.29% to 82.45%) did not show any increase in serum urea and creatinine (Group B). Medians and mean concentrations of serum urea and creatinine in 23 infected dogs are presented in Table 1.

Comparison of TT4, FT4, and cholesterol concentrations in groups A and B did not show statistically significant differences (Table 2). Correlations between concentration of TT4 and parameters of azotaemia (serum creatinine and urea concentrations) were high ($0.5 \leq |r| < 0.7$), negative, and statistically significant (Table 3). Correlation between FT4 and creatinine was average ($0.3 \leq |r| < 0.5$), negative, and statistically significant, and correlation between FT4 and serum urea was average ($0.3 \leq |r| < 0.5$) and negative but statistically insignificant (Table 3).

Table 1

Concentration of TT4, FT4, cholesterol, urea, and creatinine in 23 dogs infected with *B. canis*

Parameter	Mean	S.D.	Median	25%–75%	Min., Max.	Ref. interv.
TT4 ng/mL	8.66	2.96	8.0	6.2 – 10.4	5.0, 16.2	15 to 40
FT4 ng/dL	0.814	0.209	0.8	0.65 – 0.96	0.52, 1.33	1 to 4
Cholesterol mg/dL	248.1	51.3	233	201 – 298	179, 336	128 to 360
Serum urea mg/dL	96.13	91.41	45	37 – 125	18, 320	20 to 45
Creatinine mg/dL	1.98	1.41	1.3	1.0 – 2.5	0.9, 5.9	1.0 to 1.7

TT4 – total thyroxine concentration, FT4 – free thyroxine concentration, S.D. – standard deviation, 25%–75% - interval between 25th and 75th percentile, Min. – minimal value, Max. – maximal value, Ref. interv. – reference interval.

Table 2

Comparison of TT4, FT4, and cholesterol concentrations between azotaemic (Group A) and non-azotaemic (Group B) dogs infected with *B. canis* using Mann-Whitney *U* test

Comparison	<i>U</i>	P	Group	Median	25% – 75%	Min., Max.	Units
TT4 (group A vs. B)	31.5	0.0508	A	7.5	5.3 – 8.0	5.0, 11.2	ng/mL
			B	8.8	7.5 – 11.3	6.1, 16.2	
FT4 (group A vs. B)	46.5	0.3135	A	0.790	0.63 – 0.84	0.52, 1.02	ng/dL
			B	0.805	0.71 – 0.97	0.53, 1.33	
Cholesterol (group A vs. B)	58.0	0.7768	A	238	211 – 287	179, 336	mg/dL
			B	229	198 – 305	186, 328	

U – a value of *U* for Mann-Whitney *U* test, *P* – a value of *P*, TT4 – total thyroxine concentration, FT4 – free thyroxine concentration, 25%–75% - interval between 25th and 75th percentile, Min. – minimal value, Max. – maximal value.

Table 3

Correlations between creatinine and serum urea concentrations and concentrations of TT4 and FT4 in 23 dogs infected with *B. canis* (a value of $P < 0.05$ significant)

Statistics	TT4 and creatinine	FT4 and creatinine	TT4 and serum urea	FT4 and serum urea
<i>r</i>	- 0.5198	- 0.4201	- 0.5099	- 0.4058
P	0.011*	0.046*	0.013*	0.055

TT4 – total thyroxin concentration, FT4 – free thyroxin concentration, *r* – Pearson's correlation coefficient, P – a value of P; * – result statistically significant.

Discussion

In this study most of the dogs infected with *B. canis* had decreased TT4 and FT4 concentrations. This result is similar to that from South Africa in which, depending of the severity of the disease, most of the dogs infected with *B. rossi* had decreased TT4 and FT4 concentrations (9). It seems probable that the decrease in TT4 and FT4 concentrations in infected dogs resulted from overproduction of pro-inflammatory cytokines (leading to suppression of pituitary TSH secretion) and increased deiodinase activity, an enzyme, which causes transformation of thyroxin into triiodothyronine suppressing TRH production, in the hypothalamus (6).

Comparison of TT4 and FT4 concentrations in groups A and B did not show statistically significant differences. However, correlations between concentrations of these fractions of thyroxin and serum creatinine suggest that severity of azotaemia may be associated with euthyroid sick syndrome. This result showed that a decrease in TT4 and FT4 concentrations may contribute, among other factors such as anaemia, in renal ischaemia and eventually renal hypoxia. The question is: does the level of thyroxin only reflect the severity of the disease or also contribute to it? Thus, it seems that there is a need for further study on the benefit of thyroid hormone supplementation in euthyroid dogs infected with *Babesia*. In general, thyroxin supplementation is not recommended in euthyroid sick syndrome because of prolonged TSH suppression (5). Moreover, in humans supplementation of thyroxin showed lack of beneficial effect (2). However, the result of this work point at extremely low level of thyroxin in some dogs. Moreover, Schoeman *et al.* (9) showed association between the level of thyroxin and mortality.

This study confirmed the hypothesis that euthyroid sick syndrome may be associated with the levels of serum urea and creatinine. However, it is possible that TNF α may play the main role in the development of hypotension and decreased renal blood flow, and euthyroid sick syndrome only reflected overproduction of this cytokine (14). Thus, further studies on beneficial effects of thyroid hormone supplementation in canine babesiosis, as well as studies on the factors contributing in renal injury in dogs infected with *B. canis* are needed.

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