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Pharmacokinetics of Total Thyroxine after Repeated Oral Administration of Levothyroxine Solution and its Clinical Efficacy in Hypothyroid Dogs

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Background: Oral levothyroxine (L-T_4) supplementation is commonly used to treat hypothyroid dogs.

Objectives: Investigate the plasma profile and pharmacokinetics of total thyroxine (tT_4) after PO administration of a L-T_4 solution and its clinical efficacy in hypothyroid dogs.

Animals: Ten dogs with naturally occurring hypothyroidism.

Methods: After hypothyroidism diagnosis and supplementation with L-T_4 solution PO q24h at 20 μg/kg BW for minimum 4 weeks, the plasma profile and pharmacokinetics of tT_4 were determined over 34 hours and the clinical condition of the dogs was evaluated.

Results: Before dosing for pharmacokinetic evaluation, mean tT_4 concentration was 23 ± 9 nmol/L. L-T_4 was absorbed rapidly (t_{max}, 5 hours), reaching a mean maximal tT_4 concentration of 56 ± 11 nmol/L. The apparent terminal half-life was 11.8 hours. Clinical signs of hypothyroidism improved or resolved in all dogs after 4 weeks of treatment. The dosage of 20 μg/kg PO q24h was judged appropriate in 5 dogs, and 4 dogs required slight increases (9–16%). Twice daily treatment, with a 30% increase in dosage, was necessary for 1 dog.

Conclusions and Clinical Importance: The pharmacokinetics of L-T_4 in hypothyroid dogs was similar to that reported in healthy euthyroid dogs. Clinical and hormonal responses to L-T_4 solution were rapid in all dogs. The starting dosage of 20 μg/kg PO q24h was suitable for maintenance supplementation in 50% of the dogs, minor dosage modification was required in 4 other dogs, and treatment q12h was required in 1 dog.

Key words: Canine; Hypothyroidism; Plasma profile; Thyroid hormone supplementation.

Hypothyroidism is one of the most common endocrine diseases in dogs,[1,2] and treatment requires lifelong daily supplementation with levothyroxine (L-T_4). Poor owner compliance is a common cause of treatment failure; thus any simplification in either the treatment schedule or administration could improve owner compliance. Once-daily L-T_4 supplementation facilitates compliance and was shown to appropriately control hypothyroidism in most dogs.[3,4]

After initial treatment of hypothyroidism for 4–8 weeks, the dosage regimen should be adjusted individually, based on changes in clinical signs and tT_4 and thyroid-stimulating hormone (TSH) concentrations.[1,5,6] Optimal dosage and frequency of supplementation vary because of variability in L-T_4 absorption and biological half-life in dogs.[7] The dosage recommendations are primarily empirical, although supported by total thyroxine (tT_4) pharmacokinetic data in thyroidectomized and euthyroid dogs.[7,8] However, no data have been published on the pharmacokinetics of L-T_4 after PO administration to dogs with naturally occurring hypothyroidism. This is important because the pharmacokinetics of L-T_4 may differ between diseased and healthy dogs. Furthermore, endogenous thyroxine secretion in euthyroid dogs may affect evaluation of the pharmacokinetics of tT_4 after exogenous L-T_4 administration. Finally, concomitant evaluation of individual pharmacokinetic profile data with the clinical efficacy of L-T_4 supplementation is expected to provide additional rationale for the determination of optimal dosing instructions.

The objective of this study was to evaluate the clinical efficacy and plasma tT_4 profile after PO administration of a L-T_4 solution q24h at a dosage of 20 μg/kg for 4 weeks in dogs with naturally occurring primary hypothyroidism.

Materials and Methods

Dogs

The study was conducted at the Department of Clinical Sciences of Companion Animals of the Faculty of Veterinary Medicine, Utrecht University (the Netherlands). Dog owners were informed about the relevant details of the study and signed an informed consent form to confirm their participation. Data were collected using standardized case record forms.

Both newly diagnosed and previously treated hypothyroid dogs were eligible for inclusion. Hypothyroid dogs already supplemented with L-T_4 could be enrolled as long as (1) historical diagnostic data satisfying the inclusion criteria were available and (2) treatment was with a veterinary L-T_4 product at a dosage of approximately 20 μg/kg/day.

Dogs that were pregnant or lactating were not eligible for inclusion. Furthermore, dogs with a history or physical examination findings suggesting concurrent illness known to decrease thyroid hormone concentration (eg, insulin-dependent diabetes mellitus, hyperadrenocorticism, adrenocortical insufficiency, chronic renal,

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or hepatic failure) were not eligible for inclusion.[9] Dogs included were deemed free of other disease based on history and physical examination findings.

Newly diagnosed dogs could not have received any thyroid hormone supplementation or antithyroid drugs within 8 weeks of inclusion. In addition, no drugs known to decrease thyroid hormone concentrations (eg, glucocorticoids, nonsteroidal anti-inflammatory drugs, trimethoprim-potentiated sulfonamides, antiepileptics, anesthetics, sedatives, furosemide, mitotane, penicillins, androgens, dopamine)[10] could have been administered in the 6 weeks before inclusion.

At the first visit (inclusion), dogs were weighed and historical data, including previous drug treatment and disease history, were recorded. A standardized physical examination was performed, with special focus on scoring of the most frequent clinical signs of hypothyroidism (eg, overweight, obesity, apathy, exercise intolerance, alopecia, hyperpigmentation).

Blood samples, for determination of basal plasma tT_4 and TSH concentrations were collected in heparinized tubes. Then, a TSH stimulation test was performed using recombinant human TSH (rhTSH),[a] administered IV (100 μg for dogs <30 kg, 200 μg for dogs >30 kg). A blood sample for tT_4 concentration was collected approximately 4 hours later.[11–13] Finally, thyroid scintigraphy was performed: $^{99m}TcO_4^-$ was administered IV (90 MBq for dogs <15 kg, 135 MBq for dogs between 15 and 45 kg, and 160 MBq for dogs >45 kg). The uptake of $^{99m}TcO_4^-$ in the thyroid area and salivary glands was compared 45 minutes later.[14–18]

In both newly diagnosed and previously treated hypothyroid dogs, in addition to the clinical signs suggestive of hypothyroidism, the diagnosis of primary hypothyroidism was confirmed when all of the inclusion criteria were met:

1 Basal plasma tT_4 concentration < 10 nmol/L.
2 Increase in plasma tT_4 concentration of < 5 nmol/L 4 hours after rhTSH stimulation.
3 Decreased or absent thyroid gland function on thyroid scintigraphy, as assessed semiquantitatively by very low to low $^{99m}TcO_4^-$ uptake by the thyroid glands as compared to the salivary glands.

Treatment

During treatment, dogs remained at home with their owners, with no specific dietary requirements. Dogs were given L-T_4 solution[b] at 20 μg/kg PO q24h for 4 ± 1 weeks, preferably in the morning to accommodate the blood sampling schedule. The dogs were fed immediately after treatment. Adverse events observed during the treatment period were recorded.

Follow-Up Examination

Before follow-up examination after 4 ± 1 weeks, dogs were fasted overnight and approximately 24 hours had elapsed since last supplementation. Blood samples were collected to determine pre-treatment plasma tT_4 and TSH concentrations. Then, the usual dose of L-T_4 was administered and food was provided immediately. Blood samples were taken at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 24, and 34 hours postdosing to evaluate tT_4. Samples were centrifuged immediately and plasma stored at −20°C until analysis. Clinical signs of hypothyroidism were scored and the dogs were weighed.

The need to adjust the daily dosage of the L-T_4 solution was determined based on both clinical signs and pretreatment plasma tT_4 and TSH concentrations at the follow-up visit.

Assays

Plasma concentrations of tT_4 and TSH were evaluated at the Faculty of Veterinary Medicine of Utrecht using commercial homologous solid-phase chemiluminescent enzyme immunoassays (Immulite canine total T4, Immulite canine TSH).[c] Reference intervals for plasma tT_4 and TSH concentrations in euthyroid dogs are 19–46 nmol/L and <0.60 ng/mL, respectively.

Pharmacokinetic and Statistical Analysis

Each individual plasma concentration–time profile of tT_4 was evaluated using standard noncompartmental pharmacokinetic methods.[d] Maximal plasma concentration (C_{max}), time to reach C_{max} (t_{max}), area under the plasma concentration versus time curve (AUC, calculated using the linear trapezoidal rule from pretreatment to the last sample associated with a quantifiable tT_4 concentration), the apparent clearance (Cl_{oral}), and the apparent half-life ($t_{1/2}$) were calculated. Actual tT_4 concentrations were used; these were not corrected using pre-treatment results.

Statistical analysis (Wilcoxon test for nonparametric comparisons) was performed[e]. The level of significance was set at $P < .05$.

Results

Twelve client-owned dogs suspected of primary hypothyroidism were selected. Two dogs did not meet the inclusion criteria: tT_4 increase of >5 nmol/L after rhTSH stimulation and substantial $^{99m}TcO_4^-$ uptake by the thyroid glands. The 10 dogs included were of various breeds (3 retriever type, 3 crossbred, 4 others). Five dogs were male (2 castrated) and 5 female (3 spayed). The dogs were aged 6.8 ± 1.8 years (mean ± SD; range, 4.8–11.0 years) and weighed 27.6 ± 14.6 kg (6.8–58.5 kg) at inclusion. Nine dogs were newly diagnosed with hypothyroidism. One dog had been treated with a L-T_4 tablet[f] at 12.4 μg/kg BW PO q12h for 7 months before inclusion.

Clinical signs of hypothyroidism, as described in Table 1, had been observed for 14 months (median; range, 3–32 months) before inclusion. Metabolic signs, predominantly decreased activity or apathy and exercise intolerance, were observed in all dogs. Nine of 10 dogs were considered overweight or obese. Dermatologic abnormalities were observed in all of the dogs, with dry coat, dermal mucinosis, alopecia, hair loss, scaling, and hyperpigmentation being recorded most frequently. Other dermatologic signs, such as "rat tail," thin coat, lichenification, pruritus, dermatitis, and desquamated skin, were reported occasionally.

At inclusion, plasma tT_4 concentrations were extremely low (<2 nmol/L) in all 10 dogs. Concomitantly, all except 1 dog (0.14 ng/mL) had plasma TSH concentrations >0.60 ng/mL (1.11 ± 0.68 ng/mL). After TSH administration (TSH stimulation test), there was little to no increase in tT_4 concentrations, with post-TSH, tT_4 concentrations still <2 nmol/L in 8 of the 10 dogs, and at 3 and 5 nmol/L in the other 2 dogs, respectively. In all 10 dogs, $^{99m}TcO_4^-$ uptake by thyroid glands compared to salivary glands was scored very low to low.

Table 1. Clinical signs at inclusion and after 4 weeks of treatment with levothyroxine.

	Inclusion	Follow-up
Metabolic signs		
Overweight/obese body condition	9/10	7/10
Decreased activity/apathy	7/10	1/10
Exercise intolerance	6/10	1/10
Increased appetite	2/10	2/10
Decreased appetite	2/10	0/10
Dermatologic signs		
Dermal mucinosis	9/10	3/10
Alopecia	8/10	5/10
Hair loss	6/10	4/10
Scales	6/10	3/10
Dry coat	5/10	4/10
Hyperpigmentation	4/10	5/10
Pyoderma	3/10	1/10
Otitis externa	3/10	1/10
Neuromuscular signs		
Stiff gait	5/10	2/10
Limb weakness	2/10	1/10
Head tilt	1/10	0/10

Treatment

Dogs were treated with L-T_4 for 26–42 days before pharmacokinetic evaluation. The L-T_4 dosage at inclusion was 20 ± 1 μg/kg BW (18–22 μg/kg) PO q24h. The actual dosage at follow-up was 21 ± 2 μg/kg (19–25 μg/kg) based on body weight loss over the 4-week treatment period. Compliance with treatment, based on owners' reports, was 100% in all except 1 dog in which 1 dose was omitted in the week preceding follow-up.

At follow-up, the pretreatment tT_4 concentration (median, 25 nmol/L; range, 18–34 nmol/L; before dosing and pharmacokinetic evaluation) was significantly increased compared to the concentration at inclusion ($P = .008$). This was observed in all except one of the dogs; the tT_4 concentration was <2 nmol/L on both occasions in this dog. The pre-treatment plasma TSH concentration (median, 0.13 ng/mL; range, <0.03–1.27 ng/mL) was significantly lower ($P = .018$) than at inclusion. In 2 dogs, TSH concentration was still high (0.66 and 1.27 ng/mL, respectively). In the remaining 7 dogs, TSH concentrations were within the reference interval. The previously treated dog showed the largest decrease in plasma TSH concentration (from 2.52 to 0.25 ng/mL).

After 4 weeks of treatment, metabolic signs of hypothyroidism (exercise intolerance, decreased activity) were observed in only 2/10 dogs (Table 1). Average body weight loss was 5.3% (1.5–10.9%). Seven of 9 dogs still were considered overweight or obese. There were no clinically relevant changes in rectal temperature and heart rate.

Dermatologic signs also improved. Dermal mucinosis improved substantially, but was still mildly present in 3/10 dogs. Alopecia, scales, and hair loss also improved, and coat density was reported to have generally improved during treatment. In 1 dog, hair loss and alopecia were judged to be worse. In the dog treated previously, the dermatologic signs had resolved, but transient hair loss was reported once during the 4-week study. Otitis externa had improved in this dog and had resolved in 2 other dogs.

During the treatment period, polydipsia was reported in 1 dog that drank approximately twice as much as before inclusion. Pruritus also was reported for this dog by its owner. Concomitant treatment for otitis externa[g] was prescribed for 1 dog.

Pharmacokinetic Evaluation

The individual plasma concentration profiles of tT_4 over 34 hours post-treatment are shown in Figure 1. The pharmacokinetic parameters are summarized in Table 2.

The profiles were remarkably similar in 9/10 dogs, with plasma tT_4 concentrations starting to increase within 3 hours after L-T_4 administration. In 1 dog, plasma tT_4 concentrations were very stable, ranging from 20 to 28 nmol/L, except for a peak concentration (41 nmol/L) late at 24 hours posttreatment, and neither Cl_{oral} nor $t_{1/2}$ could be estimated reliably. All of the pharmacokinetic parameters for this dog were excluded from statistical analysis because it was an outlier.

C_{max} of 41–72 nmol/L (mean, 56 ± 11 nmol/L) was observed at 5 hours (median) posttreatment (range, 1.5–10 hours). Plasma concentrations of tT_4 regained pretreatment values in 7 dogs by 24 hours postdosing, and in 2 dogs by 34 hours post-dosing. Apparent $t_{1/2}$ was 11.8 hours (harmonic mean). A short apparent $t_{1/2}$ (4.2 hours) was calculated for 1 dog and tT_4 concentrations were very low (at or below 2 nmol/L) from 24 hours post-dosing. Apparent $t_{1/2}$ ranged from 4.2 to 48 hours, and 10 to 48 hours when that dog was excluded.

After treatment, based on clinical signs and plasma tT_4 and TSH concentrations, 5 dogs remained on the same L-T_4 dosage (21–23 μg/kg BW). In 4 dogs, slight dosage increases (9–16%) were made. One dog was changed to twice daily supplementation, concomitantly with a slight increase in the daily dosage to 26 μg/kg BW.

Discussion

The results showed that the pharmacokinetics of L-T_4 administered PO to dogs with naturally occurring hypothyroidism evaluated in this study is comparable to that in euthyroid dogs reported in the literature.[8] The combination of 3 diagnostic elements used (evaluation of tT_4 and TSH concentrations,[1,19,20] response to TSH stimulation[20,21] and thyroid scintigraphy[14]) provides strong evidence of primary hypothyroidism in the studied population. All 10 dogs had very low plasma tT_4 concentrations at inclusion, thus endogenous thyroxine secretion did not affect the pharmacokinetic evaluation of tT_4.

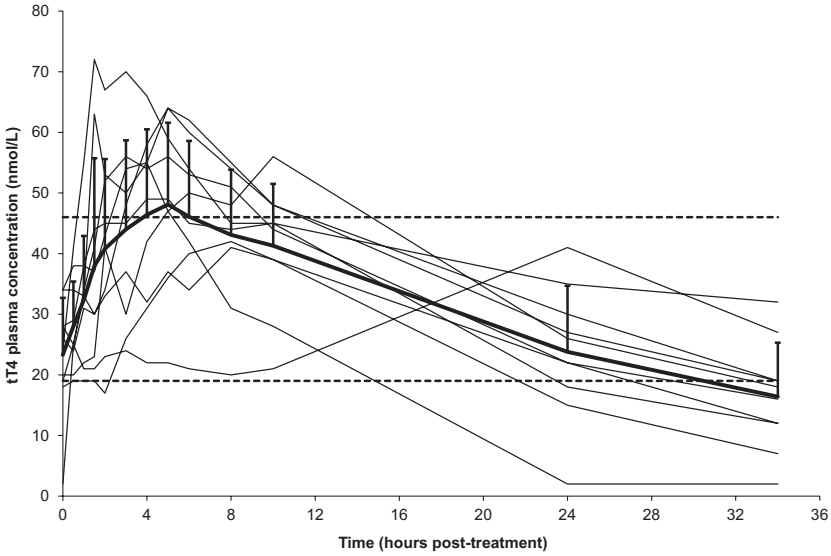

Fig 1. Individual (thin lines) and mean ± SD* (bold line) plasma tT_4 concentrations after single PO administration of 20 μg L-T_4/kg to hypothyroid dogs (n = 10). Dogs received 20 μg/kg L-T_4 PO once daily for 4 weeks before pharmacokinetic evaluation. The dashed lines represent the reference range for euthyroid dogs (19–46 nmol/L). *One canine data excluded from mean and SD calculations. tT_4, total thyroxine; L-T_4, levothyroxine.

Table 2. Total thyroxine pharmacokinetic parameters calculated after PO administration of levothyroxine (L-T_4) solution (20 μg/kg BW) to hypothyroid dogs.

Parameter	n	Value[b]
C_{max} (nmoL/L)	9[a]	56 ± 11
t_{max} (hour)	9[a]	5 (1.5–10)
AUC (nmol/h/L)	9[a]	1,087 ± 238
Cl_{oral} (L/h/kg)	9[a]	0.0213 ± 0.0096
$t_{1/2}$ (hour)	9[a]	11.8 ± 10.5 (4.2–48)

[a]Data from 1 dog not included in calculations (outlier pharmacokinetic profile).

[b]Arithmetic mean ± SD for C_{max}, Cl_{oral} and AUC, harmonic mean ± pseudo-SD (range) for $t_{1/2}$, and median (range) for t_{max}.

The mean apparent $t_{1/2}$ of tT_4 of 11.8 hours was comparable to that in healthy fed dogs (14.1 hours) reported earlier.[8] It also was consistent with values reported in thyroidectomized fasted dogs receiving 22 or 44 μg L-T_4/kg once daily[7] (9.0 and 11.5 hours, respectively). Although dogs were fed immediately after dosing, C_{max} values (56 ± 11 nmol/L, 5 hours post-dosing) were close to those reported in thyroidectomized fasted dogs receiving 22 μg/kg L-T_4 in a tablet form (C_{max} of 58 ± 23 nmol/L, 4 hours postdosing).[7]

Highly variable absorption of L-T_4 has been reported in healthy[8] and thyroidectomized dogs.[7] In our study, in an attempt to minimize variability, dogs were all fed immediately after each dose. However, the variability reported was in the same range as reported in previous work. Food was mentioned to have a possible negative effect on absorption.[8] Although interactions between L-T_4 and some food constituents (eg, walnuts, liver, albumin, soybean) have been identified in humans,[22,23] in dogs the pharmacokinetics of L-T_4 solution appear not to be influenced by the type of food, as shown recently.[24] Thus, the variability reported here and in other studies seems to originate from differences in L-T_4 absorption inherent to individuals.

Plasma tT_4 concentrations were within the reference interval in 7 dogs 24 hours after supplementation, and this was still the case in 4 dogs 34 hours postdosing. This finding is in agreement with clinical observations that this L-T_4 solution at a dosage of 20 μg/kg once daily, was effective in most hypothyroid dogs.[3] In that study, clinical signs of hypothyroidism improved or resolved in 91% of the dogs after 4 weeks of treatment at this dosage and this dosage was suitable for once daily maintenance in 79%, with 30 μg/kg required for 15%, and 10–15 μg/kg for 6%. The peak tT_4 concentration (4–6 hours after dosing) of 51 nmol/L (median) reported in that clinical efficacy study was very close to our results (46 ± 14.1 nmol/L at 4 hours, 46 ± 12.5 nmol/L at 6 hours).

The pharmacokinetic profiles of tT_4 in hypothyroid dogs in this study provide pharmacological evidence, in addition to clinical data published earlier,[3] that once daily dosing of L-T_4 solution is an appropriate treatment for spontaneous primary hypothyroidism in dogs. This dosing schedule could improve treatment compliance and thus decrease apparent treatment failure. After 4 weeks of treatment at 20 μg/kg, 90% of the dogs showed clear improvement of clinical signs as well as restoration of tT_4 and TSH concentrations to within the reference interval for euthyroid subjects. Our study also showed that activity, a metabolic sign, improves rapidly, often within the first week of treatment, whereas improvement in dermatologic signs may

take several months.[25,26] Activity was interpreted by the dog owners and is subjective, but nonetheless important. The activity level of 1 dog normalized despite a low (<2 nmol/L) tT_4 24 hours posttreatment. Hair loss increases after beginning treatment, because large numbers of hairs in the telogen stage of the hair cycle are shed.[26,27]

One dog had a pretreatment plasma tT_4 concentration <2 nmol/L, and tT_4 concentrations were below the reference range from 16 hours postdosing. This confirms the large inter-individual differences in the absorption, metabolism and half-life of L-T_4[7] and also indicates that optimal dosage and frequency of administration need to be titrated to suit the individual dog. The apparent half-life was particularly short (4.2 hours) in this exceptional case, requiring twice daily administration of L-T_4 solution.

This study offered the opportunity to follow tT_4 concentrations from 0 to 34 hours post-treatment. For 9/10 dogs, once daily administration was appropriate. Seven dogs had a plasma tT_4 concentration within reference interval for 24 hours. Nine dogs had tT_4 concentrations in the upper half of the reference interval or higher for 24 hours. This suggests that once daily administration using this L-T_4 solution is sufficient to treat most dogs with primary hypothyroidism. The dosage remained unchanged in 5 dogs and was increased by 9–16% in 4 dogs. One dog showed good clinical response after 4 weeks of treatment, and its tT_4 concentrations were within the reference range for 34 hours. However, the TSH concentration was high (1.27 ng/mL) and a dosage increase was advised conforming to the recommendations of Dixon et al[4] who stated that the maintenance of an increased TSH concentration is a reliable predictor of an increased therapeutic requirement. The effect of the dosage adjustment cannot be confirmed, because no data were collected after the pharmacokinetic evaluation.

In conclusion, the pharmacokinetics of L-T_4 in hypothyroid dogs and healthy euthyroid dogs is comparable. After repeated PO administration of a L-T_4 solution, at a dosage of 20 μg/kg once daily, tT_4 peaked at approximately 56 nmol/L at 5 hours. L-T_4 was cleared relatively rapidly, with an apparent half-life of approximately 12 hours. L-T_4 solution PO q24h controls the clinical and hormonal aspects of hypothyroidism in most dogs.

Footnotes

[a] rhTSH, Thyrogen; Genzyme Europe BV, Naarden, the Netherlands

[b] Leventa L-thyroxine sodium 1 mg/mL; MSD Animal Health, Boxmeer, the Netherlands

[c] Diagnostic Products Corporation, Los Angeles, CA

[d] WinNonlin version 5.3 software; Pharsight Corporation, Mountain View, CA

[e] SPSS version 16.0 software; IBM Corporation, Armonk, NY

[f] L-thyroxine: Forthyron 200; Eurovet Animal Health, Bladel, the Netherlands

[g] Aurizon; Vétoquinol, 's-Hertogenbosch, the Netherlands

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Conflict of Interest Declaration: G. Le Traon, S. Burgaud, and L.J.I. Horspool are employees of MSD Animal Health Innovation or Intervet International, both being subsidiaries of Merck/MSD Corporation. MSD Animal Health Innovation supplied to the Faculty of Veterinary Medicine of Utrecht the test product (Leventa L-thyroxine sodium 1 mg/mL). However, the animal and analytical phases of the study were conducted under the entire and independent responsibility of the personnel of the Faculty of Veterinary Medicine of Utrecht (including authors B.D.A.M. van de Meulengraaf and H.S. Kooistra).

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