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Urinary Iodide level in dogs and cats from different geographical areas of Hungary

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Table of contents

Introduction

Thyroid function

The thyroid glands can be found in all vertebrae animals. They are responsible for the production, storage and release of thyroid hormones, namely thyroxine (T4) and triiodothyronine (T3), as well as calcitonin -a hormone important in calcium metabolism. (Cooper and Ladenson, 2011)

The canine and feline thyroid glands are a non-palpable structure located at the mid-cervical region of the trachea, caudal to larynx. The gland is composed of a right and a left lobe running on either side of the lateral trachea. The gland is well vascularized, principally supplied by the cranial and caudal thyroidal arteries. (Feldman *et al.*, 2015)

Histologically, the thyroid gland is composed of two important endocrine cells; the follicular cells and the parafollicular cells (C-cells). The follicular cells are polarized cuboidal epithelial cells, together forming the thyroid follicles, a hollow sphere of follicular cells containing a lumen filled with a proteinaceous colloid. The colloid contains a protein called thyroglobulin, which is an important protein for the synthesis and storage of the thyroid hormones (Molina, 2013). Some of the hormone production takes place in the follicular cells, however the vast majority takes place in the colloid.

The parafollicular cells, which are responsible for the production of calcitonin are slightly larger and found in the interstitium between the thyroid follicles (Feldman *et al.*, 2015).

The synthesis and secretion of the thyroid hormones are mainly regulated by the hypothalamic-pituitary-thyroid axis, which is an extra-thyroidal negative-feedback system. Thyrotropin releasing hormone (TRH) is synthetized in the hypothalamus. TRH is then transported via axons to the median eminence and further via the portal capillary plexus to the anterior pituitary, where it binds to specific TRH receptors. This stimulation results in the release and synthesis of thyroid stimulating hormone (TSH). TSH is released into the bloodstream and act on the thyroid gland by increasing the iodine trapping, secretion and release of thyroid hormones, as well as increasing the cellularity and vascularity of the gland

itself. To maintain endocrine homeostasis, the release of thyroid hormones is regulated via a negative feedback system. Thyroxin (T4) is deiodinized into T3 by deiodinase. T3 also act on the hypothalamus to decrease the TRH synthesis. Glucocorticoids, dopamine and somatostatin also negatively regulates the TSH synthesis. (Yen, 2001; Gottschalk *et al.*, 2011)

Intra-thyroidal regulation also exists. An example of intra-thyroidal regulation is the Wolff-Chaikoff block, which explains how the iodinization of the thyroglobulin and the synthesis of thyroidal hormones decreases when iodine is found in excess, probably to protect the organism from massive T3 and T4 release in the event of excess dietary iodine intake. Another example is how the T3/T4 ratio is increased in times of iodine deficiency, since T3 contains less iodine than T4 (Riviere and Papich, 2009; Feldman *et al.*, 2015).

T3 is 3-10 times more potent than T4, thus having a greater biological effect. Nevertheless, the thyroid gland secretes 4 times more T4 than T3 into the circulation. Small amounts of reverse-T3 (a virtually inactive isomer of T3) and other deiodinated metabolites are also secreted. Most of the circulating thyroid hormones are bound to thyroid binding proteins – only 0.1% of total T4 and 1% of total T3 is found unbound in the plasma of an euthyroid dog or cat. T4 may be considered a pro-hormone, as most of it is deiodinited into the more potent T3 by enzymes present in the target tissues. This extra-thyroidal deiodinization accounts for 40-60% of the total T3 in the dog.

The thyroid hormones play a major role in the metabolism, necessary for their optimal function. The thyroid hormones regulate a wide range of processes including growth, brain development and metabolism. There is a well-known correlation between the thyroid function and reproductive performance in humans (Glinoer, 2004; Poppe, Velkeniers and Glinoer, 2007). Hypothyroidism has been linked to a wide range of reproductive disturbances in humans, such as menstrual disturbances and sometimes anovulation resulting in infertility as well as abnormal foetal development and abortion. There is evidence of significantly higher prevalence of autoimmune thyroidal disease among infertile women compared to parous age-matched women. Thyroid hormone receptors are expressed by the oocytes, which indicates that a deficiency of thyroid hormones could impair fertility (Zhang, Carrillo and Darling, 1997; Chen *et al.*, 2017).

In dogs, studies investigating the correlation of thyroid function and reproduction are limited and results are conflicting, but hypothyroidism has been observed to play a role in irregular oestrous cycles and infertility, as well as having a negative effect on pregnancy, neonatal development and perinatal survival (Peter, Gaines and Smith, 1989; Segalini *et al.*, 2009). Breed predisposition indicates that some thyroidal diseases, such as lymphocytic thyroiditis and certain types of congenital hypothyroidism, are heritable thus it would be advisable not to breed such individuals (Johnson, 2002; Wikstrand, 2011).

In two consecutive studies, hypothyroidism was experimentally induced in mixed breed bitches by the use of radioactive iodine to evaluate the reproductive effects both on short and long term. The bitches were mated and each dog produced three litters each. After the second mating, the hypothyroid bitches were treated with levothyroxine for at least 24 weeks to obtain serum TSH within reference range and serum total T4 >35nmol/L before the third mating. The results revealed that the pups born to the untreated hypothyroid bitches had significantly increased incident of perinatal mortality and markedly lower body weight compared to the control group and the treated hypothyroid bitches. There was no difference in interestrus interval, gestation duration, breeding behaviour, interval between birth of pups nor the serum progesterone concentrations at any breeding between or within groups (Panciera, Purswell and Kolster, 2007; Panciera *et al.*, 2012).

In a recent study, the thyroxin concentration, as well as progesterone was measured in pregnant and non-pregnant bitches and bitches during abortion. In this study, they did find a correlation between the thyroid function and P4. In the first week of pregnancy, there was a positive correlation between the T4 concentration and the P4 concentration in the pregnant group, whereas in the abortive group there was an opposite correlation between these two parameters. It was concluded that even if none of the dogs in this study showed any clinical signs of hypothyroidism, the bitches in the abortive group failed to respond to the increased thyroid hormone requirements during the second part of pregnancy, which resulted in abortion. This is interesting because there might be a detectable alteration in the thyroid function in a forthcoming abortion well before any other clinical signs (Thuróczy *et al.*, 2016).

Absorption of iodine

Iodine (I_2) is a crucial component of the thyroid hormones. It is estimated that iodine composes 58% of the total weight of human T3 and 65% of T4s'. The principal source of iodine is derived via food and water, either as iodine, iodinated amino acids (including T3 and T4) or short chain iodopeptides (Miot *et al.*, 2015).

The majority of the dietary iodine (I_2) is reduced to iodide (I_1^-) prior to the absorption in the small intestines. Virtually all of the iodide ingested is absorbed by the intestines and subsequently by the thyroid gland, which removes about 20% of plasma iodide at every circulatory passage (Pochin, 1950). The thyroid gland is the most iodide abundant tissue in the body, with a concentration greater than 20 to 50 times compared to plasma (Berson and Yalow, 1955). The thyroidal iodide clearance is influenced by the current status of the thyroid, meaning that the clearance is greater in the event of iodine deficiency and decreased if iodine is already present in excess. Excess iodide is excreted mainly via the kidneys, but also salivary glands, stomach and breast milk. A small amount of iodine is excreted via sweat and expired air (Miot *et al.*, 2015).

Sodium Iodide Symporter (NIS), is located at the basolateral plasma membrane of the thyrocyte and is responsible for the iodide transport from the blood stream into the thyroidal follicular cell. The NIS utilizes facilitated transfusion by transporting two Na^+ ions down its electrochemical gradient, which releases energy allowing one I^- to be transported simultaneous actively against its electrochemical gradient. To maintain the driving force of sodium, Na^+-K^+-ATPase transports Na^+ out from the thyrocyte in an active fashion (Miot *et al.*, 2015).

The iodide is then transported passively across the apical plasma membrane into the follicular lumen. Previous studies have suggested that Pendrin (Royaux *et al.*, 2000; Yoshida *et al.*, 2002), a protein which acts as a transporter of anions and is expressed in organs such as the thyroid, kidney and inner ear (Royaux *et al.*, 2000; Gillam *et al.*, 2004) is responsible for this passive transport, but there has not been any direct demonstration of this mechanism and its role in iodide transport in the thyroid gland has been questioned (Taylor *et al.*, 2002). Anoctamin-1/TEM 16 A, a calcium-activated chloride channel has been suggested to play

the role previously thought to belong to Pendrin, although this has not yet been ascertained (Twyffels *et al.*, 2014).

Production of thyroid hormones

Quickly after the free iodide enters the follicular lumen, it is oxidized by H_2O_2 via the enzyme thyroperoxidase (TPO) to obtain an iodinating form. It is then bound to tyrosyl residues of the thyroglobulin at the boundary between the apical plasma membrane and the lumen to form iodothyronine residues. Depending on the number of iodide attached to each tyrosyl residue, monoiodotyrosine (MIT) or diiodotyrosine (DIT) are formed. The following reaction is also catalysed by TPO. In this last anabolic reaction of the synthesis of the thyroid hormones, coupling of the iodothyrosine residues occur. This results in the formation of the T3 and T4, although the newly formed iodotyrosyls are still attached to the thyroglobulin molecule via peptide linkage. Which of T3 and T4 is synthesised is dependent on upon which ones of the precursors are used as substrate (Virion *et al.*, 1985; Virion *et al.*, 2005).

One MIT coupled together with one DIT will form one T3, and two DIT combined will form one T4. This reaction is utterly dependent on the structure of the thyroglobulin molecule (Lamas and Taurog, 1977) but the available iodine also plays an important role. More iodine will result in increased ratio of DIT/MIT and T4/T3, whilst the opposite will happen in the event of iodine deficiency. An exception of this is seen in the Wolff-Chaikoff block, in which the iodinization of the thyroglobulin and the synthesis of thyroidal hormones is decreased when to organism is overexposed of iodine, probably to protect the organism from massive T3 and T4 release in the event of excess dietary iodine intake (Riviere and Papich, 2009; Feldman *et al.*, 2015).

After the coupling, the mature thyroglobulin molecule contains MIT, DIT, T4 and T3 and is stored in the lumen of the follicle. The molecule is then taken up by a follicular cell via endocytosis and then transferred into lysosomal compartments, where proteolytic cleavage from the peptide bonds are performed to yield free T4 and T3. Small amounts of reverse T3 (3,3′5′-T3) which is a virtually inactive form of T3, is also formed (Miot *et al.*, 2015).

The newly formed thyroid hormones are then, by poorly understood mechanisms (Rousset and Mornex, 1991), transferred out of the lysosomal compartment into the cytoplasm of the

thyroid cell, followed by release into the blood stream. About 10% of T4 is deiodinated into T3 by type I and type II iodothyronine 5´-deiodinase before leaving the thyroid. (Salvatore *et al.*, 1996) This quota is increased upon TSH stimulation. (Laurberg, 1976) Most of the remaining MIT and DIT are deiodinated via the NADPH-dependent enzyme DEHAL1, (Gnidehou *et al.*, 2006; Moreno *et al.*, 2008) which permits recycling the iodide to the intrathyroidal iodine pool to prevent unnecessary loss of iodine to the circulation. The iodine reabsorbed into the intracellular iodine pool by this recycling mechanism exceeds the daily intake of iodine from serum to the thyrocytes by a factor of 3-5, and is thus crucial to maintain the high iodide content of the thyroid (De Groot, 1966).

Some thyroglobulin is also transferred out in the blood stream (Hjort, 1961; Roitt and Torrigiani, 1967). In case of increased thyroidal activity, increased amounts of thyroglobulin are found in the blood. This can be a useful tool in the diagnosis hyperthyroidism, as well as other, more alarming causes of increased or disturbed thyroid activity, such as hyperplastic thyroid and some forms of thyroglobulin-producing neoplasms (Miot *et al.*, 2015).

The two main regulators of thyroid activity are the iodine availability and the TSH. Iodine deficiency typically results in an increased T3/T4-ratio at first. The rationale behind this is that T3 contains one less atom of iodide than T4. A drop in T3- and T4 levels results in an increase in TSH, which in turn stimulates the activity of the thyroid gland. In prolonged deficiency, where the thyroid gland is unable to respond to TSH-stimulation by increasing hormone production, goiter develops. Excess iodine, on the other hand, may, depending on amount, acutely inhibit the hormone production to prevent a massive hormone release (Wolff-Chaikoff block), or increase the hormone production, specifically increasing DIT and T4 ratios (Miot *et al.*, 2015).

TSH influences all stages of thyroid hormone synthesis and release resulting in more iodine available for hormone production and an increased number of thyroid hormones being synthetized and released into the blood, especially T3 (Miot *et al.*, 2015).

Thyroid hormones in the plasma

Essentially all of the thyroid hormones are bound to plasma proteins during transport through the blood stream; only a very small fraction is found free in the plasma directly available to the tissues. Thyroid binding globulin (TBG) transports the majority of the hormones, but small fractions are transported with other proteins, including transthyretin and albumin. The physiological reason to why the hormones are carried with proteins rather than remaining free in plasma is not clearly understood; studies have proven that large fluctuations in transporting proteins have little impact the level of thyroidal hormone available to the tissues (Refetoff, 1989). It has been proposed that the bound hormones make up an extra-thyroidal reservoir with the capacity to maintain hormone homeostasis for some time even if there are large fluctuations in hormone secretion. It is estimated that a complete block of thyroid secretion for 24 hours only results in a 40% decrease of T4 and 10% decrease of T3. In the absence of these binding proteins, especially TBG, the levels of free hormones would become severely depleted in a matter of hours. It has also been speculated that, since the hormone-protein complex is much larger than the small hormones alone, its macromolecular properties limits unnecessary urinary loss of T4 and T3 (Chan, Besser and Landon, 1988).

In order to execute their action, the thyroid hormones must first be taken up by the cells across their plasma membrane. Since the thyroid hormones are small lipophilic compounds it was long thought that the hormones were taken up by cells via passive diffusion, but in the recent years it has been concluded that diffusion only play a minor, if any, role in the cellular uptake (Hennemann *et al.*, 2001). Instead, certain carrier proteins have been identified, which have proven to be relatively specific to T4 and T3 uptake. These include several members of the organic anion transporting polypeptide family. The OATP1C1 show particular affinity and specificity of T4 and rT3 and is most importantly expressed in astrocytes (Pizzagalli *et al.*, 2002; Bernal, 2007). Two types of proteins belonging to the monocarboxylase transporter family, MCT8 and MCT10 are also important for cellular uptake of thyroid hormones and are expressed in various tissues (Naito and Nishimura, 2008). MCT8 appear to have a higher affinity of T4 whereas the opposite is true for MCT10 (Friesema *et al.*, 2006, 2008).

Metabolism of thyroid hormones

As mentioned earlier, significantly more T4 relative to T3 is secreted by the thyroid gland. Under normal conditions, only 20% of circulating T3 is directly secreted from the thyroid, whereas the remaining 80% of T3 origins from deiodination of T4 (Gereben *et al.*, 2008). T4 is mainly a pro-hormone and must therefore be transformed into the bioactive T3 in the peripheral tissues prior to binding to its nuclear receptor initiating cellular action. This process may occur in an autocrine manner, where both production and action of T3 take place in the same cell, or in a paracrine manner, where these two processes take place in different cells (Visser, 2016). Paracrine mechanisms are especially important in the brain, where the conversion to T3 take place in the astrocytes in order to act on the neurons (Heuer *et al.*, 2005; Bernal, 2007). As well as activating mechanisms, mechanisms for inactivation of thyroid hormones are also required. These processes are carried out mainly via deiodination, but the thyroid hormones are also metabolized via other pathways including glucuronidation, sulfation, oxidative deamination and ether bond cleavage (Visser, 1996; Wu *et al.*, 2005). The details of these activating and inactivating mechanisms are further described in the paragraph below.

There are three enzymes which are involved in the catalytic reaction resulting in the deiodination of the iodothyronines. They all belong to one selenoprotein family, each containing a selenocysteine residue in its active centre (Berry, Banu and Larsen, 1991). These are called D1, D2 and D3 iodothyronine deiodinases. Deiodination of T4 can either be carried out on the outer ring or the inner ring of the molecule. Outer ring deiodination (ORD) of T4 results in the formation of T3, which is the active form of the thyroid hormones, whereas inner ring deiodination (IRD) forms the inactive metabolite rT3. T3 and rT3 are then further metabolised, mainly through IRD and ORD respectively, both resulting in the metabolite 3,3'-T2. D1 and D2 both express an ORD, which renders these enzymes capable of converting the prohormone T4 into the bioactive T3, as well as degrading the inactive metabolite rT3 into 3,3'-T2. D1 is capable of both ORD and IRD activity, whereas D2 is only capable of IRD-activity. D3 acts as an inner ring deiodinase (IRD) and is only involved in the inactivation of thyroid hormones by degrading T4 to rT3 and T3 to 3,3'-T2. (Visser, Visser and Peeters, 2017)

D1 is most importantly found in the liver, but also in other organs such as the kidneys and thyroid gland. It is believed that its ORD activity in the hepatocytes is an important contributor to the clearance of rT3 from the circulation, but D1 also participates in the production of plasma T3 from T4. Its IRD activity have a very high affinity towards sulphated T4 and T3, resulting in their degradation. In the presence of sulphated iodothyronines, its ORD activity on T4 is inhibited (Visser, 1994). Since D1 show such particular affinity towards rT3 and iodide containing residues, it is suggested that this enzyme plays an important role in the recycling of iodide, which may be used to build new thyroid hormones (Schneider *et al.*, 2006).

D2 is mainly expressed in the brain but also anterior pituitary gland and skeletal muscle (Reed, Ann and Zavacki, 2012), and express pure ORD activity. It main role is to preserve a physiologic level of T3 in tissues, regardless of changes in the concentration of circulating thyroid hormones (Schneider *et al.*, 2006). Thus, its expression can adapt in response to alterations in thyroid state.

D3 is most abundant in the brain and only have IRD activity; its role is consequently limited to the inactivation of thyroid hormones, especially T3. By degrading T3 into its inactive metabolite, the tissues are protected against excessive plasma T3 concentrations (Tu *et al.*, 1999; Bianco and Kim, 2006; Reed, Ann and Zavacki, 2012). D3 is up-regulated during pregnancy, where it is particularly expressed in the uterus, placenta and foetal tissues since high T3 levels in certain stages of pregnancy impair the normal growth of these tissues. Certain types of vascular tumours increase the expression of D3, leading to consumptive hypothyroidism as excessive amounts of thyroid hormones are degraded (Huang *et al.*, 2000).

In hypothyroidism, the T4 levels are too low. In order to secure T3 homeostasis in the brain, the expressions of D1 D2 and D3 are modified; D1 and D3 activities are down-regulated, with a net result of increased amounts of available T4, whereas D2 activity is up-regulated, enabling an increased conversion of T4 into T3 in the brain. However, in severe iodine deficiency the T4 levels may become extremely low. In this case the enzymatic adaptations described above may be unable to compensate for the deficiency. The opposite enzymatic adaptations occur in case of hyperthyroidism(O'Mara *et al.*, 1993; Burmeister, Pachucki and StGermain, 1997).

Glucuronidation and sulfation belongs to the so-called phase II detoxification reactions. In order to increase the water solubility of the hormones, resulting in facilitated urinary or biliary clearance, their phenolic hydroxyl group is conjugated with either sulphate or glucuronic acid. This conjugation also render the hormones inactive (Visser, 1996; Wu *et al.*, 2005). Only small amounts of sulphated iodothyronines are found in the circulation, since D1, which show particularly high affinity towards sulphated iodothyronines, rapidly degrade these compounds via its IRD activity (Visser, 1994). However, during certain conditions e.g. fasting and specific phases in foetal development, D1 activity is inhibited, resulting in increased levels of T3 sulphate in the plasma (Peeters *et al.*, 2005; Wu *et al.*, 2005). It is believed that the sulfation only forms a temporary hormone inactivation, forming a reservoir still available to be recovered by the action of various sulfatases before the irreversible degradation via D1 occur (Visser, 1994, 1996; Wu *et al.*, 2005). Iodothyronine glucuronides are largely excreted in the bile. In the intestines, bacterial B-glucuronidases hydrolyses a part of these compounds back into iodothyronines again, which may be recycled back into the circulation.

Ways of iodine detection

Urinary iodine correlates with iodine intake (Rasmussen, Ovesen and Christiansen, 1999) as approximately 90% of dietary iodine is excreted via the kidneys (Gibson, 2005), making urinary iodine a sensitive marker of recent changes in iodine intake. Methods for measuring iodine in urine has been used extensively to monitor iodine status on population levels due to its low cost and availability (DeMaeyer, Lowenstein and Thilly, 1979). Casual urine samples are easy to obtain, and, provided that the sample is prevented from outer iodine contamination, the iodine level can be measured with high accuracy. As long as evaporation is avoided, there is no specific time frame in which analysis is required, nor is the addition of any preservatives. The samples can be stored frozen for longer periods as long as the sample is completely de-frozen before analysis (DeBenoist, Burrow and Schultink, 2007).

A single urine sample cannot accurately describe an individuals' iodine status, since, depending on the amount if ingested iodine, the amount of iodine excreted via the kidneys varies slightly day-to-day; some fluctuations of its excretion may even be noticed within a given day (Rasmussen, Ovesen and Christiansen, 1999). However, these variations have

been showed to even out within populations, as long as a sufficient number of urinary samples are collected. By using the median as a measure of iodine distribution in a population, within-person variation can be compensated (DeBenoist, Burrow and Schultink, 2007).

There are several methods to analyse urinary iodine. Already in 1937, Sandell and Kolthoff described the reaction which occur when the yellow colour of cerium turns colourless in the presence of arsenic and iodide. It was stated that iodide act as a catalyst in the above-mentioned reaction, and by measuring the time interval of the colour change, the quantity of iodine in the sample can be appreciated. However, apart from iodide, other elements such as osmium, chloride and bromide also act as catalysts which implemented a need of methodical refinement in order to increase its accuracy towards iodine quantification (Sandell and Kolthoff, 1937).

Throughout the years, the Sandell-Kolthoff reaction has been further developed in order to achieve enhanced accuracy of the iodine content of the sample. By adding certain reagents to the urine prior to performing the classic reaction, some of the interfering particles are removed by oxidization in a process referred to as predigestion (DeBenoist, Burrow and Schultink, 2007). The results can be measured by manual spectrophotometry or colorimetrically by the aid of a stop watch and are then plotted and interpret by comparing the result against a standard curve with a known iodine concentration (Dunn *et al.*, 1993; Samidurai, Ware and Davies, 2015).

In 1993, Dunn et al. presented chloric acid as a suitable compound to use in the predigestion process. However, chloric acid is explosive and can impose adverse health effects if not handled correctly (Dunn *et al.*, 1993). To avoid this hazardous chemical, Pino *et al* replaced chloric acid for ammonium persulfate, which is a considerable safer compound to handle. The shortcoming of this method was that a significant amount of toxic waste was produced (Pino, Fang and Braverman, 1997). To decrease the amount of toxic waste produced, Ohashi et al developed a method in which both the predigestion and the Sandell Kolthoff reaction were carried out in microplates. The microplates were placed into sealed cassettes, which prevented hazardous gases to be evaporated as well as reducing the risk of cross-contamination (Ohashi *et al.*, 2000).

Semi-quantitative methods to measure urinary iodine involves the "Fast B" method, in which the results are presented as belonging to a certain concentration interval rather than a specified quantitative number. This method is considerably faster traditional methods and does not require any high-tech laboratory instrumentation. Although the result is not precise, it is often enough to know which range the sample belong to in order to implement it to an epidemiological circumstance (Dunn. JT, Myers and Dunn, 1997).

The most accurate method for measuring the urinary iodine is the inductively coupled plasma mass spectrometry (ICM-MS). This method is more technically advanced and expensive than previously mentioned methods and are only feasible when a very precise result is required (Jooste and Strydom, 2010).

In a very recent study, researchers first measured urinary iodine in microplates by a modified Sandell-Kolthoff reaction and then re-measured the samples by ICP-MS. The aim of the study was to validate the microplate method by comparing the results with the ICP-MS method, since the microplate method it is a rapid, simple and inexpensive method to measure urinary iodine without the need of highly technological advanced equipment. Indeed, the microplate method showed to correlated well with the ICP-MS method (Haap *et al.*, 2017).

Many other methods to measure urinary iodine exists. Iodide selective ion electrode provides a rapid and accurate estimate of iodide concentration (Yabu *et al.*, 1986).

Geographical differences in iodine supply

It has been known for centuries that iodine deficiency results in poor mental and reproductive health. Throughout the history, all European countries, with the exemption of Iceland, have been affected by this disorder to some degree. Over the last few decades there has been global attempts eliminating iodine deficiency by implementing iodine supplementation, primarily by introducing salt iodization. However, as late as in 2004 the World Health Organisation estimated that 2 billion people around the world are still at risk of iodine deficiency and that 20% of these people live in Europe (Andersson, De Benoist and Delange, 2007).

Iodine is found in relatively few foodstuffs and the main source of dietary iodine differs between countries dependent on geographical conditions and cultural preferences. Oceans contain the greatest amount of iodine and it becomes concentrated in seaweed and marine fish. Diets abundant in these components are commonly seen in Japan and Iceland, which explains why iodine deficiency are rare in these populations (Andersson, De Benoist and Delange, 2007; Gunnarsdottir, Gustavsdottir and Thorsdottir, 2009; Gärtner, 2016).

In some other countries, iodine in drinking water is an important source. However, studies have demonstrated that iodine levels in drinking water can differ significantly even within countries (Pedersen *et al.*, 1999; Gao *et al.*, 2014).

Soils in certain geographical locations, especially in continental areas, are naturally deficient of iodine which results in inadequate iodine content in the crops cultured in these areas, which in turn contributes to iodine deficiency in those affected populations (De Benoist *et al.*, 2004; Rasmussen *et al.*, 2009).

Geographical difference in iodine supply in Hungary

According to a study performed in 2000, 80% of the Hungarian population was iodine deficient at the time and consumed drinking water containing too low amounts of iodine. In this study, the iodine level in drinking water in different geographical areas of Hungary was compared to morbidity and mortality data from the same areas. Although the majority of the population received an inadequate amount of iodine via the drinking water, the researchers also found that several towns did have adequate iodine content in the drinking water, which was confirmed by normal urinary iodine levels and the low frequency of goitre in those residents (Farkas *et al.*, 2000).

In 1994 and 2005, three cities; Csákvár, Budapest and Szolnok, located in eastern, central and western parts of Hungary were investigated regarding the iodine status of the population. Csákvár and Budapest were both judged to be mild iodine deficient areas after the first survey, but showed substantial improvement in the second survey. Surprisingly, urinary iodine concentration was rather high (passing 300µg/l) in a number of samples in the second survey; this could be explained by the high salt content often found in Hungarian food (Péter, Podoba and Muzsnai, 2015).

In the year of 2000, Mezősi *et. al* studied the prevalence of iodine deficiency and goitre during pregnancy in the city of Debrecen, located in eastern Hungary. This region did not have a history of iodine deficiency. The results demonstrated that more than half of the subjects participating in the study suffered from iodine deficiency, which was severe in 15.6% of the women. This group also had a high prevalence of goitre. A mild iodine deficiency was also noticed in the control group. The study also noted that the consumption of iodized salt, which is considered to be one of the main dietary sources of iodine, did not influence the urinary excretion of iodine. Multivitamin supplement on the other hand significantly increased the amount of excreted iodine. The study concluded that, since there were no concerns regarding iodine deficiency in this area prior to this study, close monitoring of iodine status of pregnant women is necessary also in populations that are believed to be iodine sufficient(Mezosi *et al.*, 2000).

Iodine supply in the pet industry

In animals, the iodine supply is dependent on two factors, iodine content in pet food and iodine content in drinking water. The so-called BARF diets (Bone and raw food ratios) have become an increasingly popular choice of dog food among owners, as these owners believe that dogs as carnivorous animals shall consume a diet containing all tissues of a carcass including meat, bone, cartilage and offal. However, these diets do not only impose an increased risk of transmission of various pathogens, but the nutritional content of these and other home-made diets are often not as balanced as the commercial dog foods (Michel, 2006; Dillitzer, Becker and Kienzle, 2011). A German study investigated the nutritional composition in 95 different BARF diets and compared the results with the recommended allowance for each nutrient. It was concluded that about half of the ratios contained inadequate amounts of iodine, and one diet contained alarmingly high amounts of iodine (Dillitzer, Becker and Kienzle, 2011).

However, commercial pet diets are not always properly supplemented. This was demonstrated in a study from Argentina, where it was noticed at the University Animal Clinic in Buenos Aires that the thyroid uptake of ^{131}I was abnormally low in several dogs which were fed commercial diets, in contrast to the dogs which were fed a home-made diet where the ^{131}I uptake was normal.

To further investigate this matter, the researchers investigated the iodine content in 8 different commercial diets available in Argentina, and found that although the total iodine content in these diets varied, all of them contained iodine well above the daily allowance.
The researchers then divided 18 equally aged puppies born from bitches which were not fed a high iodine content into three groups. Group A were fed a home-made diet, group B were fed the commercial diets which contained the highest amount of iodine of the 8 diets investigated and group C was fed a home-made diet supplemented with the same amount of iodine as group B.
The results revealed in group B and C, both the ^{131}I uptake as well as the total and free serum thyroxin were decreased whereas the urinary excretion of iodide was increased compared to group A.
It was concluded that the excessive iodine content found in some of the commercial dog diets in Argentina resulted in disturbed thyroid function and hypothyroidism, which was supported by the fact that the home-made diets containing the same amount of iodine as the commercial diet resulted in the same outcome. This demonstrates that increased emphasis should be placed on iodine supplementation in commercial diets (Castillo *et al.*, 2001).

Goals

The aim of this study was to investigate whether there are any geographical differences of the amount of iodide excreted in the urine in dogs and cats in Hungary.

Materials and Methods

A total of 85 dogs and cats participated in the study. Of these, 47 dogs and one cat were sampled at the University of Veterinary Science, Budapest during the spring of 2016. The dogs sampled at the university originated from animal shelters located at 5 different geographical areas of Hungary, namely Vác (n=23), Tököl (n=8), Ócsa (n=1), Siófok (n=7), Tatabánya (n=4) and Esztergom (n=4). The shelter dogs were scheduled for spaying at the university clinic and were healthy based on the pre-surgical examination. Upon arrival, the morning urine was collected by free catch into a clean plastic container when possible; the dogs which did not spontaneously urinate were sampled by the placement of a urinary

catheter or by manually expressing the bladder during the surgery. Post-sampling the urine were transferred into urine collection tubes by the aid of plastic pipettes. The tubes were marked with identification number, sex and date and were then frozen. The urine from the remaining dogs and cats in the study were sampled by their owners via free catch method as described above and the samples were collected at the Animal Health Centre, Budafok. These animals originated from Budapest (n=16), Dunaharaszti (n=1), Érd (n=1), Kecskemét (n=1), Keszü (n=1), Szada (n=1), Székesfehérvár (n=1) and Táborfalva (n=1). In this group there were also 15 animals where no additional data was known.

The samples were analysed after thawing in the spring of 2017 using Iodide ion selective electrodes. The Iodide ion selective electrode is capable of measuring iodine levels in a wide pH range (0-12). Nevertheless, the pH of each sample was measured by a portable pH meter prior to iodine analysis in order to minimize technical errors.

Results

The urinary iodide (UI) of all animals participating in the study showed rather large individual differences, however, the majority of the samples were between 2.79-9.13 ppm with a median of 4.87 ppm and a mean of 7.40 ppm. One sample of 79.70 ppm was exceedingly high, and since it was impossible to re-evaluate the sample it was excluded from the set due to the suspicion of a technical error.

The median UI from each city was measured and details can be found in Figure 3. Two of the cities; Ócsa and Tatabánya, represented considerably higher median of urinary iodide compared to the other cities (14.90 ppm and 15.90 ppm respectively). However, only one dog represented the whole city of Ócsa, rendering this value insignificant. The animals originating from Budapest showed the greatest variation of UI with a range between 1.07-35.70 ppm whereas the animals from Esztergom presented the smallest individual variation.

There were noteworthy differences between males and females. Male urine (median 10.60 ppm, mean 12.21 ppm) contained more than the double amount of iodide compared to that of females (median 4.46 ppm, mean 5.89 ppm). The data indicates gender differences of UI, and the students t-test yielded 0.01 which is a satisfactory prediction value.

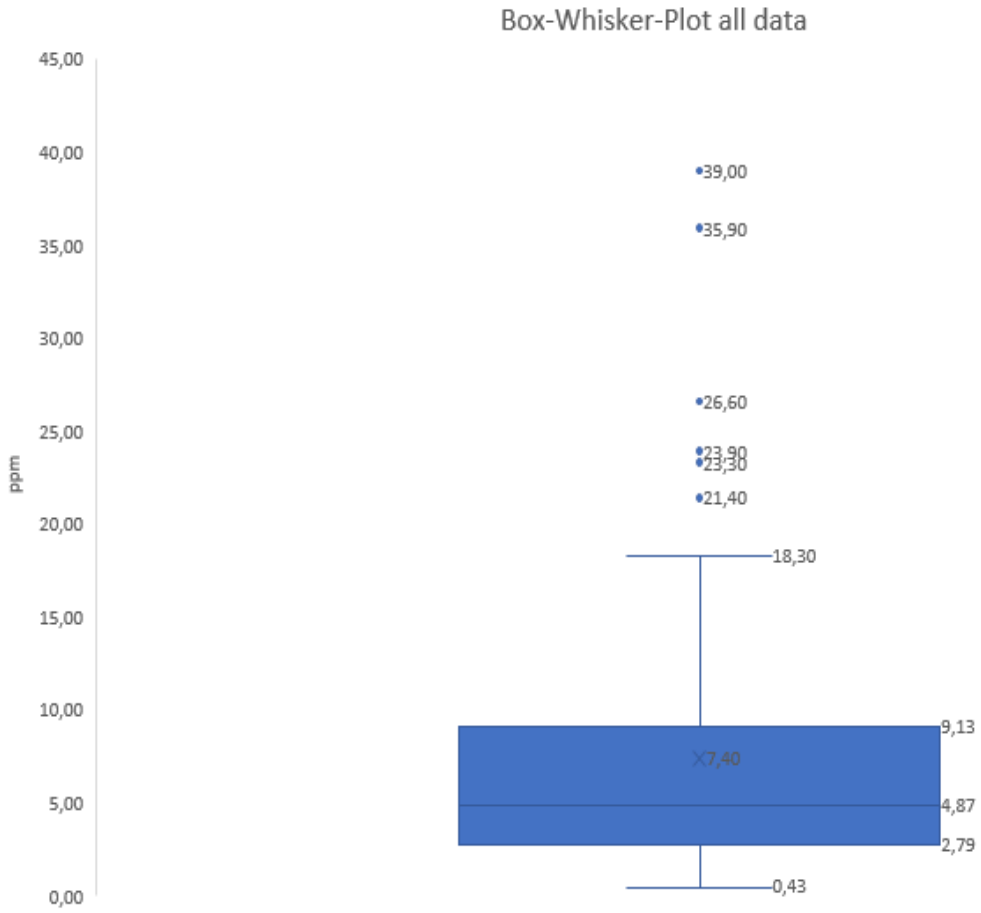

Figure 1 Box-Whisker plot of urinary iodide from all animals participating in the study, showing mean=7.40ppm, median=4.87 ppm

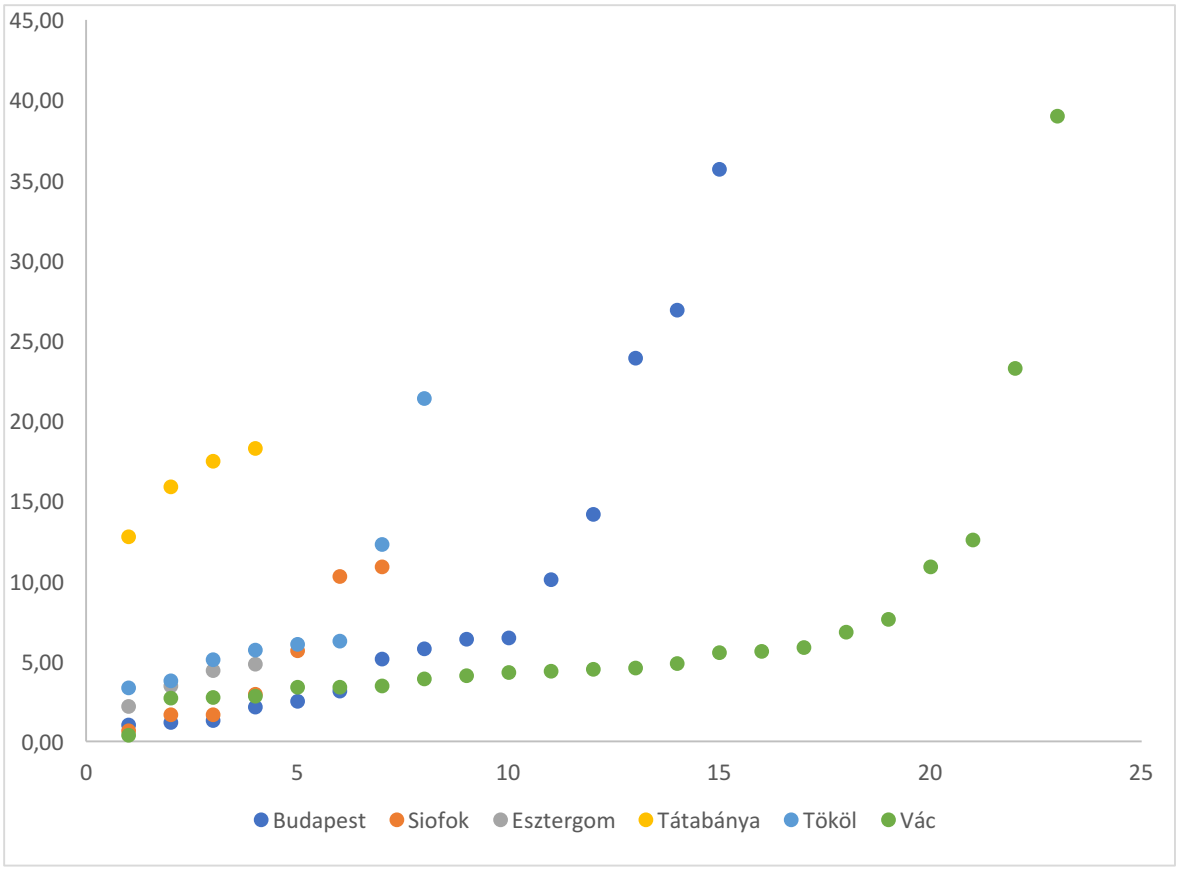

Figure 2 Urinary iodide measured in ppm from dogs living in Budapest (dark blue) Siófok (orange) Esztergom (grey) Tatabánya (yellow) Tököl (light blue) and Vác (green). Each dot represents one dog.

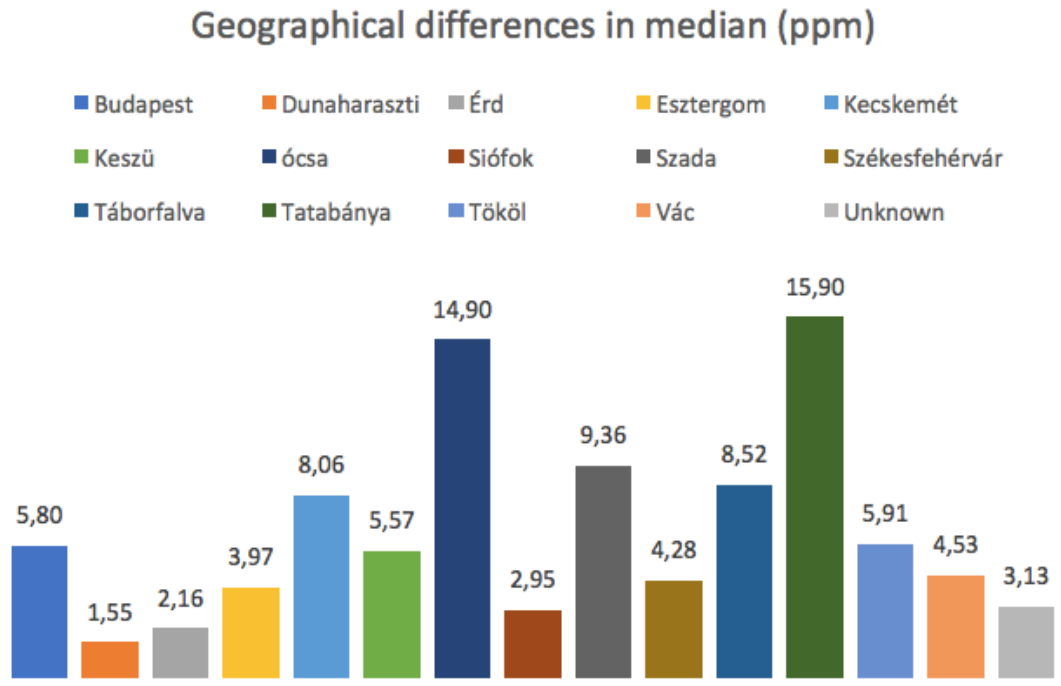

Figure 3. Median of UI in each city, measured in ppm

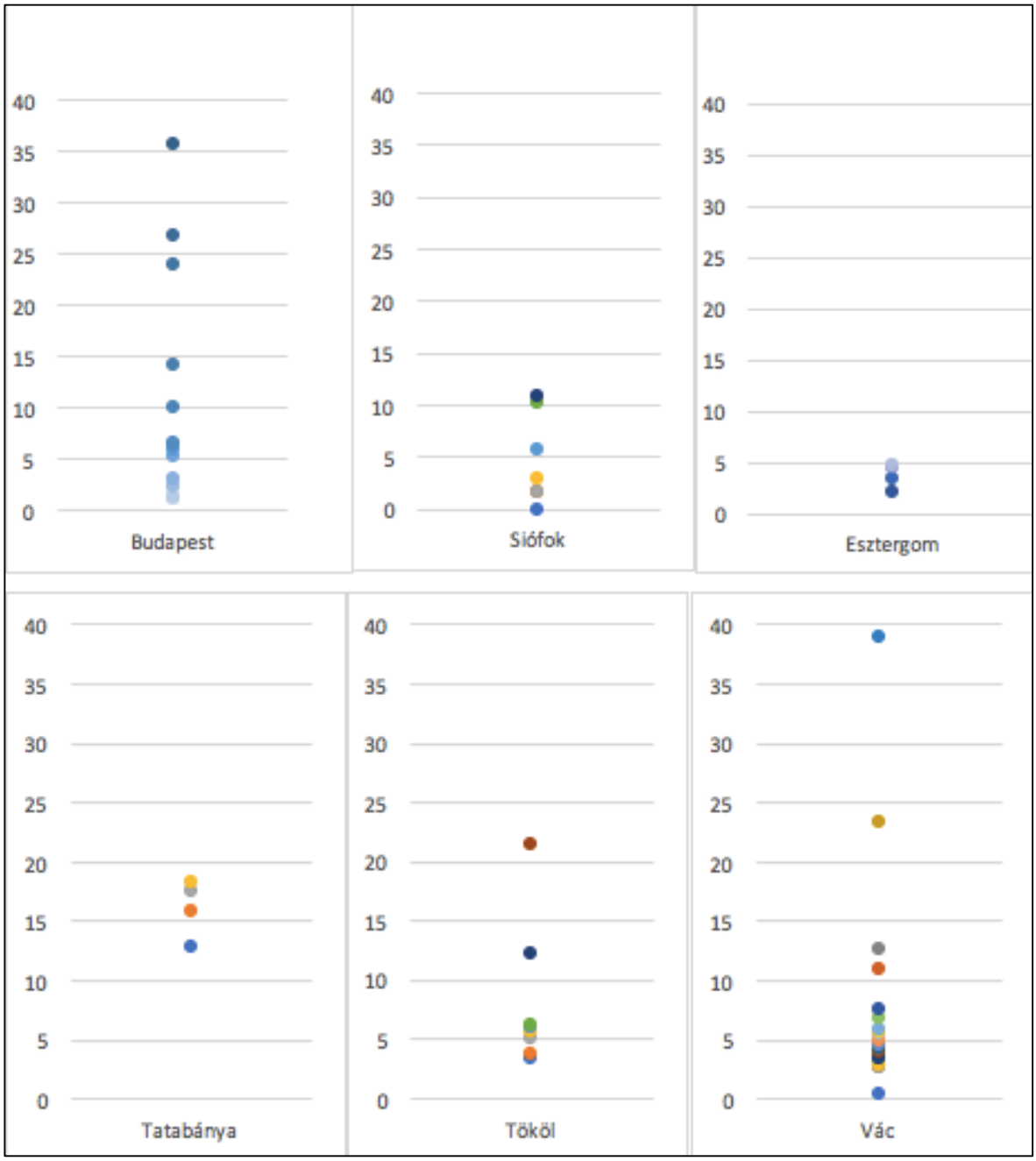

Figure 4. Individual distribution of urinary iodide of animals from six different cities

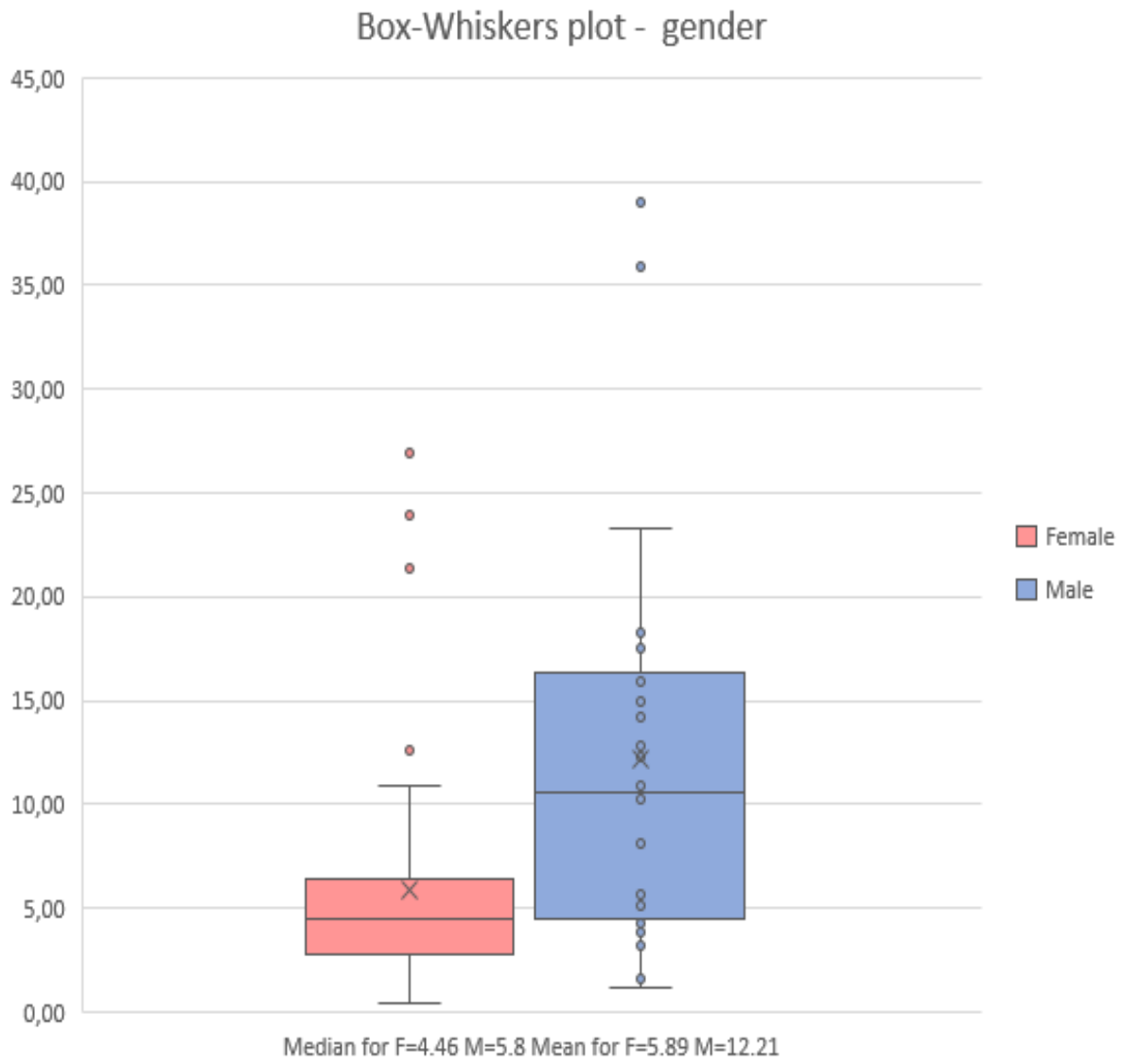

Figure 5. Box-Whiskers plot showing the differences of UI in ppm between the genders

Discussion

Although the material is too small to draw scientifically validated conclusions it is interesting to note that the results of mean and median urinary iodide presented in this study show significantly higher amounts of UI compared to the results obtained by Küblbeck. (Küblbeck, 2003) These high values are surprising, especially when considering that iodine deficiency is a relatively common condition among humans living in Hungary. A drawback of this study is that the diet of these animals was unknown. Without knowing the iodine content of the diets, it is difficult to draw any conclusions about potential geographical differences of UI that the local drinking water would have been accountable for.

The high amount of iodide in the urine of the animals from Tatabánya may have geographical reasons, but it seems more likely that the high UI is explained by the fact that all those animals were males. Gender accounts for the greatest differences of urinary iodine, which is demonstrated in Tatabánya, where all the samples derived from males and where the UI was higher with a low individual variation. On the contrary, in Esztergom, all the animas were females and the UI was quite low and there were also only minor differences of UI.

This study strongly indicates that the urinary iodine is higher in males than females, which is also supported by the student t-test which validated that $p<0.05$. This trend was demonstrated by the results seen in Tatabánya and Esztergom, in which the animals were only males and females respectively. These two cities both had a small individual variance of urinary iodine. In Tatabánya, which was represented only by males, all samples of urinary iodine were rather high compared to the rest of the cities, whereas in Esztergom, which was represented only by females, the urinary iodine level was on the lower end of the spectra. Ócsa, where only one male represented the city, showed similar amount of UI as the dogs from Tatabánya. In Vác, the two animals that showed the highest amount of UI were both males and the same was true for the animals from Siófok.

However, the similar values of UI among the animals from Tatabánya and Esztergom could have an even more simple explanation- that the animals living in these two shelters were given two different diets- one containing more and the other one less iodine, which was reflected upon the amount of iodide excreted in the urine. Although it is strongly indicated the gender play a role, diet could play an important role, that in this present data is unknown.

Future studies could measure the role of diet verses the role of drinking water on the iodine intake, by measuring the amount of iodide in the urine of dogs that consume the same commercial diet, but lives in different geographical areas, in order to estimate the role of drinking water.

Summary

Iodine is a crucial component of thyroid hormones and the urinary excretion of iodide reflects the recent iodine intake, making it a sensitive marker of current iodine status of populations. Although there has been proven correlations between iodine status and the thyroid function in humans, this matter needs to be further researched among dogs and cats. This study investigated the amount of urinary iodide among dogs and cats originating from different geographical areas of Hungary. Many of these geographical areas were represented by a very small number of animals, making it impossible to draw any conclusions regarding these different cities. The analysis of geographical differences is further complicated by the unknown effects of diet. The most remarkable result was the difference between males and females, with male urine containing almost twice as much iodide as compared to females. This preliminary study raises further questions. Future studies may continue to investigate the geographical differences of urinary iodine in dogs and cats, while taking the diet into account to see the role of iodine content of the drinking water, and also investigate whether there are any correlations between the amount of urinary iodide and the thyroid function among these animals, as well as investigating if there are any differences among urinary iodide between dogs and cats.

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