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Incidence of iodine deficiency and congenital goitre in goats and kids of Darreh Garm region, Khorramabad, Iran

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Abstract

One of the thyroid disorders of ruminants is goitre, which is triggered by iodine deficiency. This study evaluates goitre in the goats of the Darreh Garm region in the vicinity of the Khorramabad city. Three goats with congenital enlarged thyroid glands were referred to the Veterinary Teaching Hospital of the Lorestan University with signs of arrhythmia, dyspnea and anorexia. Clinical examination, radiographic and sonographic evaluations were performed. Afterward, a comprehensive visual observation was accomplished in the outbreak region and blood samples were taken for thyroid hormones measurement in does and kids. Moreover, soil and forage samples were collected to assess the iodine concentration and soil parameters. Results indicated that the thyroid hormone concentration in the serum of the affected does and kids were significantly lower than healthy and treated animals. Treatment with sodium thyroxine significantly increased the concentration of T3 and T4 hormones. Pasture (5.28 \pm 1.57 mg/kg and soil ($11.0 \pm 1.49 \text{ mg/kg}$) iodine levels were lower than normal levels in this region. Histopathological slides of the thyroid glands from the dead kids indicated thyroid follicles with different sizes and hyperplasia of the glands. Overall, a 0.5 mg/kg iodine in the diet meal of the goats needs to be considered for prevention of the iodine deficiency.

KEYWORDS goat, goitre, iodine deficiency, thyroid enlargement

1 INTRODUCTION

lodine deficiency disorders are a spectrum of disorders that can occur from the foetal stage to puberty and is a widespread cause of diseases from goitre to neurological and metabolic disorders (Constable et al., 2016). Goitre is a well-known disease caused by iodine deficiency in humans and animals. Low iodine receiving and consumption of goitrogenic agents are two reasons that may lead to iodine deficiency in animals (Clark et al., 1998). The thyroid gland is anatomically located

behind the larynx and possesses two lobes which are located lateral and medial to the trachea. lodine has a principal role in the production and secretion of triiodothyronine (T3) and thyroxine (T4) (Smith & Sherman, 2009). Thyroid hormones have vital roles in thermoregulation, metabolism and energy production, growing, reproduction health, immune system efficiency, muscle function and regulation of the circulatory system (Herdt & Hoff, 2011). Goitre is commonly detectable in lambs and kids after birth. This condition affects the survival rate of the lambs and kids due to impaired thermoregulation, decreased

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FIGURE 1 Goitrous kids with congenital enlarged thyroid glands presented to the Veterinary Hospital of Lorestan University

surfactant secretion, reduction in cardiac output, and arrhythmia (Clark et al., 1998; Singh et al., 2002). Vitamin A and selenium deficiency may be found with iodine deficiency in animals (Pearce et al., 2013). Iodine deficiency may occur in geographic areas with a lack of iodine in the soil such as mountainous regions and low-height regions far from the seas. Some environmental conditions are involved in iodine deficiency including season change and rainfall which leaches iodine from the soil (Zicker & Schoenherr, 2012). Because of possible iodine deficiency in areas with heavy rainfall, it is suggested to supplement ruminant flocks with iodine in seasons with heavy rains (Hosking et al., 1986). Among ruminants, goats mostly show signs of iodine deficiency because they eat food more selectively and consume less soil while eating (Bhardwaj & Kukovics, 2018). It has been reported that goats require more iodine in their diet compared to other ruminants. Although enough data are not available about the iodine range in the diet for goats, it is recommended that an iodine range of 0.5-0.8 mg/kg of DM of the diet should be considered for them (Nudda et al., 2009). In the present study, we reported the incidence of iodine deficiency in the Darreh Garm region, which is located in the vicinity of the Khorramabad city, Lorestan, Iran.

2 | MATERIAL AND METHODS

2.1 | Study area

Khorramabad is the capital city of Lorestan province, Iran. The city is located in a mountainous area in the Zagros Mountains and its height is 1147.8 m above sea level. This city possesses a mild and semi-humid climate with high levels of rainfall. Darreh Garm is a region around Khorramabad where the outbreak of iodine deficiency occurred.

2.2 | Case description, clinical examination and diagnostic approaches

Three goitrous kids (1 day, 28 day and 2.5 months old) of mixed breed, weighing approximately 4, 7 and 12 kg were referred to Lorestan University Veterinary Teaching Hospital with signs of the enlarged thyroid gland and anorexia (Figure 1). The owner claimed that other kids in his herd were involved. Clinical examination of the affected kids revealed some degrees of arrhythmia, dyspnea and hypothermia. Blood samples were taken from jugular veins for haematological parameters and triiodothyronine and thyroxine concentration evaluation. The kids were sent to the diagnostic imaging section of the Veterinary Hospital, and radiographs were taken in lateromedial (LM) views from the cervical region and in mediolateral and craniocaudal views from long bones. Ultrasonography of enlarged areas using Bmode imaging combined with colour Doppler flow measurement was performed from the right and left lobes of the thyroid gland after clipping of the area with 10 MHz linear transducer (esaote MyLab Seven, esaote, Genoa, Italy) for evaluation of the tissue characteristic, mass margin and vascularization. Moreover, the owner stated that some of the kids died immediately or 24 h after birth and referred a carcass to the Hospital for a necropsy. Tissue samples were taken from the hypertrophic thyroid glands and fixed in 10% neutral buffered formalin solution and sent to the department of pathology of Lorestan University for histopathological assessment. Haematoxylin and eosin (H&E) staining was performed as follows; tissue samples were embedded in paraffin blocks, sections with 5 μ m thickness were made, mounted on slides and eventually were stained with haematoxylin and eosin (H&E). Goitrous goats were treated using sodium thyroxine 0.2 mg/kg per day for 20 days (Singh et al., 2006).

2.3 | Sample collection

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The affected herds visited in their location in order to a complete assessment and also sampling from animals, soil and pasture. Blood samples were taken from kids suspected of iodine deficiency and lactating goats for measuring plasma thyroid hormones. 70 blood samples were collected from five herds (each herd seven samples from the kids suspected of iodine deficiency and seven samples from their mothers) located in various geographical regions (centre, north, south, east and west) of the study area. Furthermore, as a control group for determining the concentration of thyroid hormones in the endemic goats of the studied area, 14 blood samples were collected from does (n = 7) and kids (n = 7) that were clinically healthy. Subsequently, serums were separated from the blood samples and were utilized for thyroid hormones assessments. Pasture sampling was performed from the centre of the mentioned area and at a distance of 500 m in four areas of the west, east, north and south from the centre. Pasture samples (about 25 g each sample) were taken from 2 to 3 cm above the ground in dimensions of 1×1 m². Soil samples (200 g per sample) were taken from 0, 5, 10 and 15 cm of ground depth (using a staff gauge) in non-rocky and flat areas. Soil samples were stored in sterile non-metallic containers and transferred to the laboratory (Zarazma Mineral Studies Company, Tehran, Iran).

2.4 | Sample analysis

Serum thyroid hormones (T3, T4 and TSH) were measured by electrochemiluminescent immunoassay (ECLIA) (Roche Elecsys 2010 system, Bialystok, Poland). Automated Elecsys 2010 analyser measures T3 and T4 levels based on the competition principle and examines the level of TSH based on the sandwich principle (Kazerouni & Amirrasouli, 2012).

Soil texture was obtained using the hydrometric method, soil acidity was obtained from saturated mud using a pH meter and electrical conductivity was obtained from saturated mud extract using an electric conductivity meter (Bordbar & Mortazavi Jahromi, 2006). The soil samples were then kept at 105°C for 24 h and then passed through a 2 mm sieve and a total of 50 g was sent to the laboratory to determine the iodine concentration. Inductively coupled plasma optical emission spectroscopy (ICP-OES) method was employed to measure iodine concentration (Nölte, 2021). Forage samples were washed using distilled water and subsequently dried at 60°C and powdered. Afterwards, samples were ground in the laboratory using an agate mortar to a particle size of fewer than 100 μ m, then iodine concentration was measured by ICP-MS method (Inductively coupled plasma mass spectrometry) (Kalra & Maynard, 1998).

2.5 | Statistical analysis

At first, the distribution of soil, forage and blood data was evaluated by using the Shapiro Wilk test. Therefore, normally distributed data (control and treatment) were analysed using an independent sample *t*-test



FIGURE 2 Map of the Khorramabad city and Darreh Garm region in which outbreak has occurred

and in non-normal distributed data Mann–Whitney test was applied. In order to determine the differences in the mean between kids and goats and control animals in different geographic regions were performed using a Kruskal–Wallis test. In terms of the post hoc analysis, linear contrasts were constructed to analyse the differences in mean values between affected, treated and control groups at each region using the Duncan test. All data were analysed using the MedCal statistical software program (Version 19.6, Ostend, Belgium) and the level of significance was recorded at p < 0.05.

3 | RESULTS

The study area is shown in Figure 2. As can be seen from Figure 2, the Darreh Garm region is located in the north of the Khorramabad city with an 80 km² area. Radiographs of the referred kids to the Hospital revealed an enlargement with soft tissue opacity in front of the trachea (Figure 3a). Additionally, radiographs of long bones did not show any abnormality. Plain sonography in B-mode showed a mass with heterogeneous echogenicity compared to adjacent structures, and relatively well-defined, with miniature hyperechoic lines lateral to the trachea with the size of $3.09 \text{ cm} \times 1.74 \text{ cm}$ (Figure 3b). Colour flow Doppler mode in longitudinal and sagittal planes indicates the blood supply of the thyroid gland (Figure 3c). Pasture and soil samples were collected from the centre, north, south, east and west of the study region. Results for iodine concertation in the pasture of the Darreh Garm region are presented in Table 1. The iodine concentration of the east sampled pasture was 7.23 mg/kg which was the highest level among the rest of the samples, and the sample from the north with a concentration of 3.26 mg/kg was the least level of the iodine. Physicochemical properties, composition and iodine concentration of soil are represented in





FIGURE 3 (a) Radiographic evaluation of the goitrous kid which indicated an enlargement in the cervical region with soft tissue opacity. (b) B-mode sonography of the mass revealed a Heterogeneous echogenicity, sagittal (SAG) size was 1.74 cm and anteroposterior (AP) was 3.09 cm. (c) Colour flow Doppler-mode sonographic appearance of the mass indicated the blood supply. Total volume (TV) of thyroid lobe (right lobe) was 1.76 cm and venous (V) volume was 4.9 ml

TABLE 1	lodine concertation in pasture of different geographical
regions of Da	rreh Garm

Sampling region	lodine concentration (mg/kg)
Centre	6.10
North	3.26
South	4.17
East	7.23
West	5.65
Mean	5.28 ± 1.57 (3.33-7.24)
GNR (mg/kg DM) ¹	0.5-0.8
MAA ²	50

Total concentration (mean \pm SD with 95% CI in parenthesis). GNR, goat nutritional requirement (mg/kg dry matter); MAA², maximum allowable amount of iodine in forages.

¹NRC 2007.

²NRC 2005.

Table 2. Soil iodine concentration of the west region was the highest level (12.65 mg/kg) and the lowest level of soil iodine was for the north region (9.23 mg/kg). Levels of T3, T4 and TSH in doe of five geographical regions before and after treatment are shown in Table 3. Based on Table 3, levels of the T3 and T4 before treatment were significantly lower than the control group (p < 0.001). Moreover, the level of T3 and T4 between treated goats and non-treated goats was significant (p < p0.001). No significant difference was observed for TSH levels between non-treated goats and the control group (p > 0.05). Thyroid hormone levels in kids of five geographical regions before and after treatment are shown in Table 4. There was a significant difference between nontreated groups and the control groups for T3 and T4 hormones (p < p0.001). T3, T4 and TSH levels significantly increased in the treatment groups compared to the non-treated groups. Histopathological slides of the thyroid glands from the dead kids indicated thyroid follicles with different sizes and hyperplasia of the glands. Tall columnar epithelium with decreased colloid, disruption of normal thyroid follicular

TABLE 2 Physicochemical properties, composition and iodine concentration of soil in different geographic regions of the Darreh Garm and

Parameter	Centre	North	South	West	East	Total
Electrical conductivity	0.46	0.56	0.38	0.49	0.39	0.46 ± 0.07 (0.36-0.55)
Acidity	7.98	7.65	7.89	7.92	7.88	7.9 ± 0.12 (7.71-8.02)
Clay (%)	16.28	19.25	16.52	12.43	14.62	15.8 ± 2.52 (12.7–19.0)
Sand (%)	56.29	52.23	55.30	60.30	56.17	56.06 ± 2.9 (52.5-59.6)
Silit (%)	28.44	27.22	28.26	27.25	29.65	$28.16 \pm 1.00(26.92 29.41)$
Organic carbon (%)	0.79	0.83	0.72	0.76	0.81	0.78 ± 0.04 (0.73-0.84)
Lime (%)	57.66	60.45	56.44	50.23	49.61	54.9 ± 4.76 (48.9-60.8)
lodine concentration (mg/kg)	10.52	9.23	10.11	12.65	12.43	11.0 ± 1.49 (9.13-12.84)
Organic materials (%)	1.54	2.12	1.86	1.42	0.97	1.58 ± 0.44 (1.04–2.13)

TABLE 3 Levels of T3, T4 and TSH in doe of five geographical region before and after treatment

Parameter	Before treatment ($n = 35$)	After treatment ($n = 35$)	Control group ($n = 7$)	Reference intervals (Paulíková et al., 2011)
T3 (ng/dl)	$101.83 \pm 9.91^{a,b}$	142.97 ± 8.47	162.13 ± 6.11	144.00-162.00
T4 (μg/dl)	$2.28 \pm 0.40^{a,b}$	5.35 ± 0.67	5.37 ± 0.26	4.96-6.16
TSH (μIU/ml)	0.011 ± 0.005	0.014 ± 0.002	0.09 ± 0.097	0.01-0.10

Data with normal distribution are displayed as Mean ± SD. Nonnormal distribution data are expressed as median and interquartile range.

 $^{a}p < 0.001$ compared with the control group.

 ${}^{b}p < 0.001$ compared with the treated group.

architecture, atrophy and separation of the follicles was observed in the histopathological slides (Figure 4a and b).

total concentration (mean \pm SD with 95% CI in parenthesis)

4 | DISCUSSION

340

lodine deficiency in ruminants leads to goitre, especially in newborns. lodine deficiency will affect the survival rate of the lambs and kids. Hypothermia due to iodine deficiency occurs in newborns. Moreover, owing to the cortisol-like effects of the thyroid hormones in stimulating alveolar cells to discharge surfactant, the survival rate in the lambs and kids will be reduced (Clark et al., 1998). Iodine deficiency in the foetus may impact the growth and differentiation of cells in various organs. Therefore, a dead foetus with reduced growth and congenital disorders is frequently observed in the herds with iodine deficiency (Clark et al., 1998). Several methods have been employed to diagnose iodine deficiency in herds, including thyroid weight, thyroid to body weight ratio in newborns, histological evaluation of the gland and T4 concentration in the serum of the animals (Clark et al., 1998). The present study evaluates an iodine deficiency outbreak in a region in the vicinity of the Khorramabad city, Lorestan, Iran.

Goitre results in hypertrophy and hyperplasia of the thyroid gland. It happens due to iodine deficiency in the body that leads to the overproduction of the TSH from the pituitary gland and the attempt of the thyroid to compensate for the deficiency in the thyroid hormones. In several previous studies, thyroid glands were hypertrophic in the reported lambs and kids (Bhardwaj & Kukovics, 2018; Singh et al., 2002). In agreement with the previous studies, in the present study, kids were found with the hypertrophic thyroid gland. Arrhythmia, diminished cardiac output and dyspnea have been reported in goitrous animals (Abraham et al., 1987). Kadum and Luaibi (2017) performed a study on experimentally induced hypothyroidism in goats and concluded that the pulse rate per min and the respiratory rate were significantly reduced in the group with iodine deficiency compared to the control group. Treatment with potassium iodide significantly increased the pulse rate per min and the respiratory rate in the treatment group (Kadum & Luaibi, 2017). Herein, arrhythmia and dyspnea were diagnosed in the goitrous goats which were improved after therapy.

TABLE 4 Levels of T3, T4 and TSH in kids of five geographical region before and after treatment

Parameter	Before treatment ($n = 35$)	After treatment ($n = 35$)	Control group ($n = 7$)	Reference intervals (Paulíková Seidel et al., 2011)
T3 (ng/dl)	122.81 (117.31, 129.20) ^{a,b}	224.47 (210.84, 245.36)	250.58 ± 6.07	282 ± 1.01
T4 (μg/dl)	$3.49 \pm 0.57^{a,b}$	7.62 ± 0.80	8.35 ± 0.37	8.65 ± 1.86
TSH (μIU/ml)	0.016 (0.014, 0.023) ^b	0.180 (0.160, 0.245)	0.08 ± 0.087	0.01-0.10

Data with normal distribution are displayed as Mean \pm SD. Nonnormal distribution data are expressed as median and interquartile range. ^ap < 0.001 compared with the control group.

 $^{b}p < 0.001$ compared with the treated group.

DAVOODI ET AL.



FIGURE 4 Haematoxylin and Eosin staining method for evaluation of tissue damage in thyroid gland tissue with ×100 (a) and ×400 (b) magnifications respectively. (a) Tall columnar epithelium with decreased colloid, disruption of normal thyroid follicular architecture, atrophy and separation of the follicles were detected. (b) Magnified view of epithelial cells with hyperplasia and increased epithelial layer thickness

Previous studies regarding iodine concentration of the soil revealed that the level of iodine in the soil has a wide range from <0.1-150 mg/kg in various geographical regions. The iodine level of the soil is significantly higher than rocks. The source of iodine in the soil is iodine in the atmosphere, which originates from marine iodine. Hence, geographical areas in the vicinity of seas have more iodine concentration in the soil. Another factor that impacts the iodine concentration differences is known to be the soil components (Fuge, 2013). Research on iodine levels in soils and herbage revealed that soil pH is inversely correlated with the iodine level of herbage (McGrath & Fleming, 1988). Altınok et al. (2003) have assessed iodine therapy on forage yields of alfalfa and found that forage iodine levels increased following iodine treatments (Altinok et al., 2003). In a similar previous study on goitre in goats and kids, iodine levels in soil and alfalfa hay were measured and the values were 14.67 mg/kg and 0.1 mg/kg in the soil and alfalfa hay respectively (Bires et al., 1996). Results concerning iodine concentration in the present study are in keeping with previous studies. In the present study, a proportional relationship is observed between the iodine levels in the soil and pasture and in geographical areas with higher iodine levels in the soil, a higher iodine amount was detected in the pasture as well. In consistent with the findings of the Bires et al. in which the soil iodine in the region with goitre in kids and goats was 14.67 mg/kg, in the present study the iodine concentration of the soil was $11.0 \pm 1.49 \text{ mg/kg}$.

Clark et al. (1998) examined iodine deficiency in sheep flocks. They assessed iodine pasture levels in March, June, July and August and found that pasture iodine in March and August was in the highest amount. Additionally, they declared that 0.5 mg/kg of iodine in the diet meal of sheep is required to meet their nutritional needs of iodine, and this increases to 2 mg/kg of iodine when pasture includes goitrogens (Clark et al., 1998). In another study, the concentration of iodine in the pasture was evaluated by Knowles and Grace (2007). The iodine concentration was 0.15 mg/kg dry matter in the forage (Knowles & Grace, 2007). In the present study, forage samples were collected in May from five regions where the outbreak occurred, and the forage samples did not include goitrogens. Besides treatment with sodium thyroxine, the

owner was suggested adding 0.5 mg/kg of iodine in the diet meal of goats.

Campbell et al. (2012) evaluated the goitre outbreak in northeast Victoria. Radiographic evaluation of the long bones of the lambs in their study indicated a decrement in the opacity of the epiphyses. Also, cuboidal bones such as carpal and tarsal bones became rounded (Campbell et al., 2012). In contrast to the previous study, in the present research, we did not find mentioned defectives in the bones and just a mass with soft tissue opacity was observed in the cervical region.

Singh et al. (2002) have examined the levels of T3 and T4 in the serum of the goitrous goats after treatment. They found that T3 and T4 levels in the goitrous goats after therapy with sodium thyroxine significantly increased (Singh et al., 2002). Kadum and Luaibi (2017) examined levels of the T3, T4 and TSH in the goats with hypothyroidism and found that levels of T3, T4 and TSH were remarkably lower than that in the control group. In the treatment group, levels of thyroid hormones significantly increased compared to the non-treated group (Kadum & Luaibi, 2017). Singh et al. (2006) have assessed the immune status and medical management of goats in endemic goitre. They have concluded that treatment with sodium thyroxine was more effective than treatment with aqueous iodine and significantly increased total immunoglobulin and phagocytic index (Singh et al., 2006). In agreement with previous studies (Singh et al., 2002; Kadum & Luaibi., 2017; Singh et al., 2006), in the present research T3 and T4 levels in the does before treatment was significantly lower than that in the control group. Treatment with sodium thyroxine notably increased levels of thyroid hormones in the serum. Furthermore, in the treated kids T3, T4 and TSH significantly increased compared to the same kids before treatment.

Kumar Bhardwaj (2018) reported that the thyroid gland in goitrous goats had tall columnar epithelium with diminished colloids in histopathological slides (Bhardwaj & Kukovics, 2018). Ong et al. (2014) in a study on hyperplastic goitre in two adult dairy cows have found disruption of the thyroid follicles, atrophy and separation of the follicles, and alterations in the form of epithelial cells (Ong et al., 2014). In agreement with these findings, in the present study, the thyroid gland was more hypertrophic with stretched epithelium and low colloid, and some follicles were atrophied and separated.

5 | CONCLUSION

In regions with iodine deficiency in the forage and diet of goats and sheep, it is recommended that iodine be added to the diet meal to prevent congenital goitre in goats and lambs. In the present study, treatment with sodium thyroxine was able to effectively treat iodine deficiency in the herd.

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AUTHOR CONTRIBUTIONS

Farshid Davoodi: conceptualization; investigation; methodology; resources; software; supervision; visualization; writing-original draft; writing-review & editing. Amir Zakian: data curation; formal analysis; investigation; methodology; project administration; writing-review & editing. Alireza Rocky: validation; visualization. Abbas Raisi: investigation; methodology; resources; writing-review & editing

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PEER REVIEW

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342