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Validation of a rapid urinary iodide test in the goat: a preliminary study

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Abstract

A rapid urinary iodide test was assessed in goats to validate its effectiveness in field conditions. The test provides a semiquantitative assessment of the iodine intake and could be valuable for animal population. Two hundred and forty-six dairy goats were randomized selected for this study. According to iodide content in urine, results were classified in three groups (<10 μg/dL, 10–30 μg/dL, >30 μg/dL). These findings were in agreement with spectrophotometrical determinations and with serum iodine values. Sensibility and specificity were 89% and 78%, respectively. Data on reproducibility and interfering substances were also valuable. Given the technical simplicity, cost-effectiveness and its stability in heat environmental conditions, the rapid urinary iodide test should be considered as a valuable method for epidemiological surveys in field conditions or in development countries.

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1. Introduction

Iodine deficiency disorders (IDD) are a major public health problem worldwide and cause goiter, an increased incidence of stillbirth, abortions, and congenital abnormalities, including endemic cretinism in human population (Dunn, 1992; Maberly et al., 1994; WHO, 1996). Thyroid diseases are well known in companion animals but less so in livestock, in which nutritional iodine deficiencies have been of greater importance, particularly in iodine deficient areas of the world (Keen and Graham, 1989).

The main route of excretion of iodides is by the kidneys, through which almost all the iodide that was not trapped by the thyroid gland is lost in urine. A small but significant amount is lost in the saliva, and minimal amounts are lost in tears, feces, sweat, and milk. In ruminants, a significant amount may be lost in feces as well (Kaneko, 1997). However, iodide loss through the feces has not been estimated. Urinary iodine analysis is the most common biochemical

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method used for assessing the iodine status of human populations (Dunn et al., 1993) but there is great diversity in available methods with respect to cost, technical sophistication, sample processing capacity, and performance (May et al., 1997). Techniques that avoid problems of having to destroy potential interfering substances, such as neutron-activation analysis or inductively coupled plasma-mass spectroscopy, are generally considered gold standard methods for urinary iodine analysis but are not widely used because of high cost and degree of sophistication (Binnerts and Das, 1974).

In livestock production there is a need for relatively quick, simple and cost-effective methods to determine urinary iodide concentration in a high number of samples. The purpose of this work was to assess in goats a urinary iodide test (Rendl et al., 1998), which is easy to perform, does not require instrumentation or apparatus and has been tested under different conditions of temperature and storage.

2. Materials and methods

Urinary iodine is usually expressed in μg/dL, rather than in SI units (μmol/L). Because of World Health Organization guidelines for IDD status use μg/dL, we express our results in μg/dL (conversion factor: 1 μg/dL=0.079 μmol/L; 1 μmol/L=12.7 μg/dL).

The test was provided by Merck KgaA (Frankurter Strasse 250, D-64271, Darmstadt, Germany) and consists of the following materials and reagents:

- Disposable columns, 65×10.5 mm, packed with purified activated charcoal (Merck patent number WO 96/27794) for removing interfering substances; column support; three test cups (25×50); and color scale (pictogram).
- Buffer solution (citrate-hydrochloric acid (pH 4), Merck catalog no. 1.09435); peracetic acid/H_2O_2 (dropping bottle), 1.2% in 30% H_2O_2; and 3,3′,5,5′-TMB (tetramethylbenzidine) (dropping bottle), 2.5 mmol/L analytical grade in ethanol.

Spectrophotometry using an UV/VIS Lambda 1 and 2 spectrophotometer (Perkin Elmer) at 655 nm wave length was used as reference method to determine the iodide content in urinary samples due to its high correlation with high-performance liquid chromatography (r=0.94) (Rendl et al., 1998).

The study was carried out between October 2000 and February 2001. Two hundred and forty-six goats belonging to 12 dairy farms located in the Canary Islands (Spain) were randomized selected using the cluster sampling system (Greiner and Gardner, 2000). The sample size was calculated following the standard formula described by Greiner and Gardner (2000):

$$n = \left(\frac{Z_1 - \alpha/2}{e}\right)^2 \theta(1-\theta)$$

where $Z_1-\alpha/2$=1.96, α=0.05, θ=the priori estimate of sensitivity and specificity (0.8) and e=the desired error margin on the estimate (0.05). All the selected animals were females, >6 months of age, and grazed local pasture around the farms. One hundred and twenty-four animals (farms of origin=6) were located at an altitude between 400 and 900 m (group A) above sea level. The second group (goats=122, farms of origin=6) originated at or slightly above sea level (group B). Urinary samples were taken using urethral catheterization (catheter diameter 2.0–2.2 mm) and blood samples by jugular venopunction. Each urine sample was tested within 2 h after collection, as described by Rendl et al. (1998).

The test was performed according to the manufacturer's instructions. Optical spectra were recorded at 655 nm, in a 1-cm path cell, against a water blank reflecting a high reputed correlation with HPLC (Rendl et al., 1998). The catalytic effect of iodide in the redox reaction between the colorless 3,3′,5,5′-TMB and the peracetic acid/H_2O_2 to yield colored products, is the basis of the photometric method used by the rapid urinary iodide test for detection of iodide in urine. Possible effects of interfering substances were assessed by adding known amounts of potassium thiocyanate, L-ascorbic acid, sodium sulfide, and sodium chloride to five samples of each group with determined urinary iodide concentrations to achieve a final concentration of 300 μmol/L, 20 mmol/L, 50 μmol/L and 100 mmol/L for potassium thiocyanate, L-ascorbic acid, sodium sulphide, and sodium chloride, respectively. The test was then performed with and without these substances. Serum iodine was measured in 119 randomized selected animals belonging to each urinary iodide group (Table 2) using a paired-ion,

Table 1
Urinary iodide values according to each group

Urinary iodide content group	<10 μg/dL group	10–30 μg/dL	>30 μg/dL group	Total
Group A	42	16	66	124
Group B	18	3	101	122
Total	60	19	167	246

reversed-phase HPLC, as described by Rendl et al. (1998). Reproducibility of the rapid urinary iodide test was assessed using three consecutive assays of 30 spectrophotometrically tested samples, 10 from each group.

Serum T4 was determined in 100 randomized selected animals, 50 belonging to group A and 50 belonging to group B. These sample sizes were adjusted to the recommendations of Bourdoux (1988) on iodine status in populations (50–100 samples). T4 was determined using a modified ELISA, Vet Test, IDDEX Laboratories.

3. Results

Urinary iodide contents are summarized in Table 1. Serum iodine values are presented in Table 2. Spectrophotometrical determinations are compared with rapid urinary test findings in Fig. 1. Sensitivity and specificity of rapid urinary iodide test compared with spectrophotometric measures are summarized in Fig. 2. T4 values were 6.2±1.88 and 6.7±0.95 μg/dL for group A and group B, respectively. No statistical differences were found (p=0.167). Sensitivity and specificity were 89% and 78%, respectively. A total of 48 out of 246 investigated samples showed disagree between rapid urinary iodide test and spectrophotometric values, with similar percentage in the <10 mg/dL group (11%; 7/60) and in the >10 mg/dL (11%, 41/186). On the other hand, spectrophotometric determinations did not fully discriminate between iodide concentration <10 mg/dL and 10–30 mg/dL (Fig. 1), with some marginal values from each group within the another one. Interfering substances had no effect on the initial urinary samples. Reproducibility was also valuables, all samples were within the same range value for three consecutive times. Tests were also made on aqueous solutions of potassium iodate, and no color changes were recorded.

Table 2
Serum iodine values according to each urinary iodide content group

Serum iodine data (μg/dL)	<10 μg/dL group (n=50)	10–30 μg/dL group (n=19)	>30 μg/dL group (n=50)	Total population
mean	0.5402	2.0113	3.6617	1.5954
Standard deviation	0.4096	0.8798	1.7079	0.8477
Minimal value	0.1	0.9	1.97	0.1
Maximal value	2.4	4.6	10.19	10.19

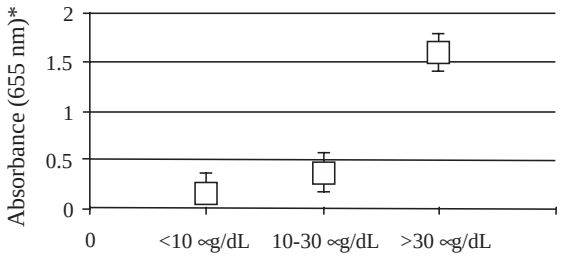

Fig. 1. Spectrophotometrical range according to rapid urinary iodide test. *: Mean±S.D.

4. Discussion

Sensitivity and specificity of the rapid urinary iodide test was 89% and 78%, respectively (Fig. 2). Tests on reproducibility or interfering substances also showed a good validity of the rapid urinary iodide test for goats. However, ammonium is one of the interfering compounds which is very important when ruminant urine is investigated. Ammonium is partially removed by the activated carbon but when high quantities are presents in urine, the color reaction could be completely suppressed (Rendl et al., 1998). Based on our results, ammonium does not seem to interfere the color reaction at the normal concentration presents in goat urine. Interfering substance tests and tests on aqueous solutions of potassium iodate indicate that the method do not detect iodine in an other biological relevant form than iodide. The test was easy to perform, did not require any technical equipment and seemed appropriate for epidemiological studies in areas where sophisticated methods are not available. We estimate that 60–80 samples can be tested within 2 h and the cost of the test ranges from 1.03 to 1.06 euros, depending on the number of kits

Urinary iodide concentration (spectrophotometer, ∝g/dL)

Urinary iodide concentration (Rapid Test, ∝g/dL)	< 10	> 10	Total
< 10	53 (89%)	7 (11%)	60 (24%)
> 10	41 (11%)	145 (78%)	186 (76%)
Total	94 (38%)	152 (62%)	246 (100%)

Fig. 2. Sensitivity and specificity of rapid urinary iodide test compared with spectrophotometric measures.

used. Moreover, the test can also be performed under conditions of heat and storage usually found in developing countries, where the test could prove to be particularly valuable. However, it should be taken into consideration that the test provides only a semiquantitative assessment of the iodine intake. Thus, results are always approximates and could be valid when examined a large number of samples, not for individual cases.

Urinary iodide has been determined in dogs (Castillo et al., 2001) and goats (Slosarkova et al., 1999). The results obtained in this study using urine samples from goats were higher than those published by Slosarkova et al. (1999) but similar to those reported for humans (Rendl et al., 1998). For human populations, The World Health Organization has proposed the cut-off for interpreting urinary iodide content of 10 mg/dL. Our results show that goats with iodide urinary levels <10 mg/dL had iodine serum values lower ($P<0.05$) than those obtained from goats with urinary values >30 mg/dL. However, although serum T_4 levels resulted lower in the group A, differences were not found with statistical significance. The lower quantities of urinary iodides detected in animals belonging to the mountain group would indicate that iodide levels in the circulation were adequate for the thyroid gland metabolism but not enough for its excretion. This could suppose that higher iodine demands derived from higher metabolic requirements could not be supplied in this group of animals. On the other hand, clinical picture related to iodine deficiency such as goiter, stillbirth or poor weight gain in juvenile animals are sometime seen in the herds located in the mountain areas. These data could indicate that urinary iodide values <10 mg/dL are also inadequate for goats, and that mineral supplementation should be provided to the diet of the animals in these areas.

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