Determination of the Occurrence of *Toxoplasma Gondii*, *Giardia Duodenalis* and *Cryptosporidium* spp. in leafy Greens in Marrakech using a Molecular Method

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Abstract

Background: The association between the parasitic illnesses and the consumption of contaminated food is more and more described. However, there is still a lack of studies investigating the occurrence of parasitic contamination in food matrices. The aim of the present study was to assess the presence of *T. gondii* and *Cryptosporidium* spp. oocysts and *G. duodenalis* cysts, in three leafy greens (coriander, lettuce and parsley) commonly consumed raw.

Methods: A total of 152 leafy green samples were collected in Marrakech from April 2018 to October 2019. Parasites were eluted and concentrated before detection of their DNA by real-time qPCR.

Results: The analysis revealed an overall rate of contamination of 32.2% (49/152), with 29.6% (45/152) positive for *T. gondii*, 2.6% (4/152) for *G. duodenalis*, while *Cryptosporidium* spp was not detected.

Conclusion: The results showed that leafy greens vegetables available in markets of Morocco are subjected to protozoan parasites contaminations. Thus, humans can be exposed to these parasites through vegetables consumption. Further investigations can be performed to acquire new epidemiological data on the health risk of these protozoan diseases in Morocco.

I. Introduction

Foodborne parasites as *Toxoplasma gondii*, *Giardia duodenalis* and *Cryptosporidium* spp. can be transmitted to humans through the accidental ingestion of infective stages in food. Foodborne sources include meat, fish, shellfish, vegetables, and fruits. These protozoan have been detected in leafy green vegetables (leafy greens) in both developing and developed countries [1–4]. Most of these leafy greens are consumed raw or slightly cooked, increasing the probability to be exposed to infective parasites that would normally be controlled by food processing temperatures [5].

Giardia duodenalis has been implicated in illness outbreaks worldwide related to the consumption of contaminated food including raw vegetables and fruits [6, 7]; *Cryptosporidium* spp. has also been identified in several outbreaks [8–10] that were mainly associated with ready-to-eat salad, sandwich containing salad, and apple cider and juice. In contrast, there have been only two reported outbreaks of toxoplasmosis associated with the consumption of fresh produce or juice [11, 12]. Some surveillance studies have been conducted worldwide for foodborne parasites (e.g., *T. gondii*, *G. duodenalis* and *Cryptosporidium* spp.) in fresh vegetables [1–3, 13], but fewer such studies have been performed in North Africa [14–19] and especially in Morocco [20, 21]. These investigations of foodborne parasites were based on different elution procedures and a variety of microscopic and molecular detection methods [22], that render difficult to compare the data. If a standard method is now available for the detection of *Cryptosporidium* and *Giardia* (oo) cysts in fresh produce (ISO18744) [23], it is not widely used mainly because of its cost (consumables and personal). Also, since parasitic (oo) cysts and leafy greens have different physical and chemical characteristics, the method appears to be not suitable for all types of vegetables [24]. In addition, this method does not allow the detection of other relevant foodborne parasites such as *T. gondii* or *Cyclospora*. It is, then, essential to have a consensual method allowing the detection of different protozoan parasites in different types of vegetables.

The monitoring of protozoan parasites in fresh vegetables involved generally three important phases – pretreatment of matrices by elution, then concentration and detection of the parasites. The efficiency of these steps conditions the final result and the estimation of contamination rates in fresh vegetables. Indeed, the elution step that aims to recover parasites from vegetables is crucial since all the subsequent steps will depend on [25]. Therefore, the use of an adequate elution buffer and an efficient isolation method is required to maximize the (oo) cysts recovery as well as the accuracy of the results [25]. In addition, the use of a fast, low-cost and sensitive detection method for parasitic monitoring is of great interest so that it can be applicable for routine controls in food industries. However, these methods can still be considered expensive in developing countries.

The present study aimed to detect *T. gondii* and *Cryptosporidium* spp. oocysts and *G. duodenalis* cysts, in three leafy greens (coriander “*Coriandrum sativum*”, lettuce “*Lactuca sativa*” and parsley “*Petroselinum crispum*”) in Morocco. Leafy greens were collected in markets in Marrakech (central Western Morocco), from April 2018 to October 2019, and were analyzed using a rapid molecular method, which involved (oo)cysts elution in 0.01% Tween 80 / PBS; pH 7.2, mechanical lysis of (oo) cysts walls and DNA extraction using the FastDNA kit, then detection by real-time qPCR.

Ii. Material And Methods

1 Parasites preparation

T. gondii oocysts (ME49 strain, genotype II) were obtained as described by Dubey [26] and stored in a 2% H₂SO₄ solution, without antibiotics at 4°C until use. Before use, oocysts were washed three times in H₂O to remove H₂SO₄.

Purified *C. parvum* oocysts (Iowa Isolate) and *G. duodenalis* cysts (H3 isolate, B assemblage) suspensions were purchased from Waterborne® Inc. (New Orleans, LA, USA) and kept in PBS at 4°C until use.

The parasitic suspensions were numbered on Kova slide (Kova® Slide 10) using a phase contrast microscope (Axioskop 40, Zeiss). Each stock solution of parasites (*T. gondii*: 27.5 x 10⁵ oocysts/ml; *G. duodenalis*: 8.6 x 10⁵ cysts/ml; *C. parvum*: 6.25 x 10⁵ oocysts/ml) was used to prepare serial dilutions in H₂O to obtain working suspensions for the following experiments.

2. Spiking experiment for determination of the limits of detection of *T. gondii*, *G. duodenalis* and *C. parvum* by real-time qPCR in leafy green vegetables

To be able to interpret negative samples in the prevalence study, the limits of detection of the used molecular method were determined for each parasite. To that aim, fresh vegetables (coriander, lettuce and parsley) were bought from local supermarkets in Reims (France) and damaged leaves and roots were removed. The leaves were then cut into pieces of about 2.5 cm x 2.5 cm x 2.5 cm, mixed and weighted to obtain samples of 25g. The leaves were placed on clean paper towels. Each sample was spiked with a serial dilution solution containing 1, 5, 10, 10², 10³, 10⁴ or 10⁵ of each parasite and deposited on leaves (in several spots of 5 µl maximum) and then allowed to dry in a microbiological safety cabinet for 2 h before being processed [27]. Contaminated samples were washed in filter stomacher bags (BagFilter® R, Intersciences) with 100 ml of 0.01% Tween 80 / PBS, pH 7.2 buffer using a horizontal mechanical shaker Promax 1020 (Heidolph) (10 min, 130 movements per min) at 37°C. The filtrates were collected and centrifuged at 3000 × *g* for 30 min. The resulting pellets were then submitted to DNA extraction and analyzed by real time qPCR as described below. All parasitic loads were tested in three replicates. The limit of detection (LOD₉₅) was defined as the lowest quantity of parasites that could be detected in at least 95 % of the positive samples.

3. Samples collection for environmental study

The sampling strategy targeted the region of Marrakech and aimed: i) to analyze leafy greens that are commonly consumed raw; ii) to analyze vegetables representing each sector of the city, including both rural and urban areas, and iii) to address the three main different markets available for vegetables in Marrakech. These markets were: i) the wholesale market, located in the industrial district and organized in sheds or sales areas; ii) an urban supermarket located in the city center “Gueliz”; iii) a rural market in “Ghmate” situated 30 km Southeast Marrakech where each vendor brings his own products or imported ones and presents them for sale. In the three markets, vegetables are displayed for the customers to choose their preferred items, touching and handling the product as they make their selection before paying at the counter. Although the samples came from different regions of the kingdom, it was difficult to determine their origin because of the lack of traceability.

Consistent with these objectives, coriander, lettuce and parsley were selected, and each of them was purchased randomly, each month from April 2018 to October 2019 (except August 2018 and July 2019) at each of the three markets, transported to the laboratory and processed within 24 h. A total of 152 samples were collected including 51 samples from the wholesale market, 50 samples from the supermarket, and 51 samples that were obtained from individual vendors at the rural market (Table I).

For each vegetable, 25g samples were prepared and processed as described above (paragraph 2), and analyzed for the presence of the three parasites by real time qPCR.

4. Parasite detection by real-time qPCR

Vegetable pellets were submitted to mechanical lysis using a FastPrep®-24 Instrument (MP Biomedical, Solon, OH) as previously described for *T. gondii* [28, 29], that generated collision movements of oocysts/cysts with three types of beads (0.1 mm silica beads, 1.4 mm ceramic beads and a 4 mm glass bead). Parasites DNA were then extracted with the FastDNA™ SPIN kit (MP Biomedicals, Solon, OH) according to the manufacturer's instructions. The detection of the three parasites was performed by real-time singleplex qPCR already characterized in terms of specificity, and targeting the 529 bp repeat region of *T. gondii* [30], the 16S-like ribosomal RNA of *G. duodenalis* [31] and a specific 452-bp sequence encoding the DNA J-like protein of *C. parvum* [32]. This latter assay is able to detect *C. parvum*, *C. hominis* and *C. meleagridis*. These qPCR assays have been already successfully used on environmental [33, 34] and vegetable samples [35]. qPCR reaction was performed for each parasite in duplicates (technical replicates) and consisted of 12.5 µl of reaction mixture (iQ™ Supermix, Bio-Rad), 1 µl of 400 nM of each primer, 0.5 µl of each probe (with a final concentration of 200 nM for *Toxoplasma* and *Giardia* and 100 nM for *Cryptosporidium*), 4 µl of H₂O, 1 µl of bovine serum albumin (BSA, 10 mg/ml) and 5 µl of DNA extract, for a total volume of 25 µl. The reactions were performed on a QuantStudio™3 apparatus (Applied Biosystems™, Thermofisher) and were divided into 2 steps; the DNA denaturation at 95°C for 3 min and amplification through 40 cycles of 15 s at 95°C and 1 min at 60°C [33]. A negative control was added to each qPCR plate to verify the absence of external contamination that could induce non-specific fluorescence, in addition to a positive control (DNA extracted from parasitic suspension) to monitor the progress of the amplification and the validity of the used reagents. The Cq value corresponds to the cycle number at which the fluorescence exceeds a fixed threshold and allows the quantification of the amount of the target DNA. A well was considered positive when the Cq value was inferior to 40 (i.e. ≥ 1 target copy/mix). In case the DNA was detected in both wells, the sample was considered positive, whereas if it was only detected in one well, a second qPCR was performed in duplicate. In this case, the sample was considered positive if at least 2/4 wells were positive.

III. Results

1 Limits of detection

The limits of detection were determined on artificially spiked samples (Table II). Overall the method allowed to reach low level of detection of *T. gondii* and *G. duodenalis* in some vegetables (0.04 (oo) cysts/g). However, variable sensitivity could be observed depending on the parasite and the vegetable. Indeed, the detection of *T. gondii* and *C. parvum* was more successful in coriander and parsley than lettuce, whereas the detection of *G. duodenalis* was more successful in parsley and similar in coriander and lettuce. The limits of detection ranged from 0.04 to 40 oocysts / g of leafy greens, with a minimum (LOD₉₅ < 0.04 (oo) cyst) for *T. gondii* in coriander / parsley and *G. duodenalis* in parsley, and a maximum (LOD₉₅ < 40 oocysts) for *C. parvum* in lettuce.

2. Occurrence of *T. gondii*, *G. duodenalis* and *C. parvum* in environmental vegetable samples

Of the 152 analyzed samples by qPCR, the overall detection rate of protozoan parasites was 32.2% (49/152); 29.6% (45/152) were positive for *T. gondii*, 2.6% (4/152) for *G. duodenalis*, and none was positive for *C. parvum/hominis/meleagridis*. The contamination rates observed in each vegetable varied (lettuce 43% (n = 22); coriander 33% (n = 17) and parsley 20% (n = 10) (Fig. I; Table III).

The most frequently detected parasite was *T. gondii*, with the highest rate (22/51) in lettuce, followed by coriander (14/51) and parsley (9/50).

G. duodenalis was detected in (3/51) samples of coriander, and (1/50) sample of parsley, while it was not detected in lettuce.

IV. Discussion

Surveillance studies from around the world reported widely varying contamination rates for the three protozoan in leafy greens, attributable to differing sampling strategies, geographic location, sanitation and detection methodologies.

Parasitic (oo) cysts have usually been monitored in food matrices following three steps: elution, concentration and detection [22]. The difference in properties of the various food matrices could make it difficult to remove and detect protozoan (oo) cysts [2]. Despite the establishment of a standard ISO [23] for the microscopic detection of *Cryptosporidium* spp. and *G. duodenalis* in leafy greens and berry fruits, there are still other protozoan parasites like *T. gondii* that need development of standardized methods. In addition, this standard is based on immunomagnetic separation technique followed by immunofluorescent assay for detection that are expensive, time consuming and require a microscopy expertise, making this method difficult to use for routine checks by food processors. Therefore, there have been a multitude of described methods worldwide for parasitic detection in vegetable matrices with different recovery efficiencies and detection levels [22]. This contributes to the under diagnosis of protozoan parasites in food.

In our study, we used a rapid molecular method that involved elution with 100 ml of 0.01% Tween 80 / PBS pH 7.2 in filter stomacher bags, under horizontal shaking at 37°C. It allowed the detachment of (oo) cysts from vegetable leaves due to the capacity of Tween 80, as a nonionic surfactant, to enter the interface between the vegetable surfaces and the parasites to ease adsorption at the interface and to minimize the interfacial tension, and consequently reduce the attractive interactions between the microorganisms and vegetables surfaces [36]. Consistent with this, 0.01% Tween 80 / PBS elution buffer has already been successfully used to elute parasites from leafy green vegetables [37–39]. In addition, the filter stomacher bags allowed removing large particles (above 250 µm) that could interfere particularly at the DNA extraction phase. The duration of elution was sufficient to ensure (oo) cysts recovery with minimum formation of debris and matrix that could affect the process of elution. The recovery of the parasitic forms could have been improved by a purification step using the immunomagnetic separation “IMS” as recommended in the standardized method ISO [23] for the detection of *G. duodenalis* and *C. parvum*. However, the IMS is time consuming, more expensive and is not suitable for the detection of *T. gondii* since the only described monoclonal antibodies for *T. gondii* oocyst walls led to a low recovery rate ranging from 0.2 to 35% and a high value of LOD (33 oocysts/g of basil and raspberries) [35]. DNA extraction is mentioned to be affected by the technique that is used to prepare the DNA template, with superiority of some techniques over others [40]. Vegetables constituents (e.g., polysaccharides, polyphenols, pectin and xylan) may be co-extracted with the targeted parasite DNA and thereafter inhibit the PCR by cross-linking with nucleic acids and modifying their chemical properties [41]. Herein, the DNA extraction was performed using a kit based on mechanical disruption of the sample that has been successfully used to extract parasitic DNA in food matrices [29, 40, 42] as well as other matrices like soil [28] and cat feces [43]. The overall method led to LOD₉₅ ranging between 0.04 and 4 parasites/g that are suitable with occurrence studies except for *C. parvum* in lettuce (LOD₉₅ = 40 oocysts/g).

Compared to *T. gondii* and *G. duodenalis*, the detection of *C. parvum* is less sensitive in our study; this could be related to the use of a single copy target gene. Shapiro *et al.* [44] recently designed a multiplex system also based on the 18S ribosomal RNA that should be tested on our samples in the future. Despite the low limits of detection of oocysts, we were not able to observe a linear response ($r^2 > 0.98$) between the Cq and the number of parasites spiked on leafy greens, probably due to the presence of inhibitors in vegetable samples. Further adjustments could include inhibition control and efforts have still to be made to overcome inhibition problems and to succeed in quantifying the detected parasites.

This study achieved its goal of determining the occurrence of *T. gondii*, *G. duodenalis* and *C. parvum* in leafy greens marketed in Marrakech, over the period April 2018 and October 2019. It is known that leafy greens can be exposed to parasitic contamination, given the nature of their foliage and the structure of their surface, for instance lettuce has broad and irregular leaves, while coriander and parsley have flat leaves and dense foliage providing a large contamination surfaces and favoring parasitic attachment. Indeed, we have detected a relatively high proportion of contaminated leafy greens (32%), similarly to the finding of our recent study undertaken in 2017, in Marrakech [20]. However, studies from some other more populated and largest countries of North Africa have indicated different levels of contamination in various leafy greens (e.g., 35.6% in Alexandria, Egypt [17]; 2.2% in Tripoli, Libya [14]). In more developed countries, the proportion of contaminated vegetables with parasites tends to be lower [e.g., less than 1% in Canada [2]; 6 % in Norway [13]].

Recently, it has become evident that ingestion of oocysts in fresh produce is an under recognized transmission route of contamination. A recent source attribution meta-analysis has highlighted the involvement of vegetables in sporadic toxoplasmosis [45]. The present study revealed a high rate of *T. gondii* (29.6%) in leafy greens. The detection of this parasite has been reported elsewhere such as in Czech Republic 9.6% (28/292) [46] and Portugal and Spain 42.9% (14/35) [47], using molecular methods. In North Africa, only two studies have been conducted to investigate the presence of this parasite in fresh vegetables: the study performed in Egypt [15] has revealed using microscopy a contamination rate of 5.6 % (19/212), while our previous study [20] showed an overall rate of 21% (18/86), using the same qPCR.

In our study, *G. duodenalis* was detected in 2.6% of leafy greens, this was in agreement with the contamination rates reported in other studies, using microscopy, as Libya with a rate of 2.2% (12/54) [14] and Egypt with a rate of 4% (2/49) [19]. In contrast, our present finding was lower than those reported recently in Morocco with 7% (6/86) [20], Egypt with 9% (47/530) [18] and India 5% (13/284) [3]. Previously, Bouhoum and Amahmid [21] have evaluated the presence of *Giardia* cysts in crops irrigated with treated and untreated wastewater, in Marrakech: this study revealed the presence of *G. duodenalis* in 20.3% of the 9 analyzed samples of coriander, while it was not detected in crops irrigated with treated wastewater. This may confirm that the use of raw wastewater for irrigation contributes to parasitic contamination.

Cryptosporidium spp. was not detected in any of the analyzed samples, while the rates observed in leafy greens in other studies were considerably higher: 20% (99/494) [16] and 4.6% (4/86) [20]. The difference between our previous [20] and present results could be due to two reasons: i) the oocysts could be present in low quantities that were considerably under the limit of detection of this described protocol, and/or ii) the observed *Cryptosporidium* spp. oocysts may not belong to *C. parvum*, *C. hominis* or *C. meleagridis* that are targeted by the qPCR used in the study.

The data presented here on the occurrence of *T. gondii*, *G. duodenalis* and *C. parvum* in leafy greens is a crucial step in identifying potential sources of parasitic infection and potential exposition of consumers in Marrakech. A limitation of molecular assays for the detection of protozoan (oo) cysts in produce is the inability to distinguish between living and dead organisms. Therefore, a positive result does not necessarily mean that there is a risk for consumers. However, populations of (oo) cysts often consist of viable and non-viable organisms in different proportions, and as very low dose of (oo) cysts are necessary to lead to human infection, any findings should be considered as an indicator of exposure.

V. Conclusion

The relative contribution of foodborne transmission in parasitic infections was poorly studied, but recent studies of meta-analysis of risk factors for parasitic infections identified the consumption of unwashed vegetables as a relevant risk factor for infection [45, 48]. In this context, we wanted to investigate occurrence of protozoan in leafy greens in Morocco. In this study, we used a molecular method to investigate the presence of *T. gondii*, *G. duodenalis* and *Cryptosporidium* spp. in marketed leafy greens in Marrakech, in complement of a previous study [20] that are the first studies in Morocco. Our results showed relatively high level of contamination (overall rate of 32.2%). This potentially exposes the consumer to the risk of contamination although vegetables are not the only vehicles of parasitic transmission to humans (waterborne transmission for example). On the other hand, the lack of traceability of vegetables in the study region (no information about the sources of irrigation water and the cultivation methods) as well as the lack of investigations of the prevalence of these pathogens in both humans and fresh vegetables make it difficult to link up the presence of parasites with parasitic diseases in Marrakech.

Declarations

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Competing interests

The authors declare that they have no competing interests.

Availability of data and material

The authors confirm that the data supporting the findings of this study are available within the article.

Code availability

Not applicable.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

All the authors agree and give consent for the publication.

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Tables

Table I Sampling details of leafy greens collected in Marrakech, from April 2018 to October 2019

Season		Spring (2018 & 2019)				Summer (2018 & 2019)				Autumn (2018 & 2019)				Winter 2018				Total
Vegetables	Origin	SM	WM	RM	Total	SM	WM	RM	Total	SM	WM	RM	Total	SM	WM	RM	Total	Total
Coriander	No. analyzed samples	5	5	5	15	4	4	4	12	5	5	5	15	3	3	3	9	51
Lettuce	No. analyzed samples	6	6	5	17	3	3	4	10	5	5	5	15	3	3	3	9	51
Parsley	No. analyzed samples	5	6	5	16	3	3	4	10	5	5	5	15	3	3	3	9	50

SM: supermarket; WM: wholesale market; RM: rural market.

Table II Limits of detection (LOD₉₅) of the qPCR to detect *T. gondii* oocysts, *G. duodenalis* cysts and *C. parvum* oocysts in artificially contaminated leafy greens vegetables

	<i>T. gondii</i>		<i>G. duodenalis</i>		<i>C. parvum</i>	
	LOD ₉₅ (oocysts/g)	Cq mean +/- SD	LOD ₉₅ (cysts/g)	Cq mean +/- SD	LOD ₉₅ (oocysts/g)	Cq mean +/- SD
Coriander	0.04	36.7 ± 1.0	0.4	30.7 ± 4.2	0.4	35.1 ± 1.6
Lettuce	0.4	33.7 ± 2.7	0.4	36.4 ± 0.6	40	33.2 ± 1.0
Parsley	0.04	35.8 ± 0.5	0.04	36.1 ± 2.3	4	37.4 ± 1.0

Table III Results of the detection of *T. gondii* and *G. duodenalis* in vegetable samples

<i>Toxoplasma gondii</i>					
Sample number	Product	Origin	Collection date	Cqm $\pm$ σ	Number of positive wells*/ Total
1	Coriander	Wholesale market	21.09.18	37.4 $\pm$ 0.0	2/2**
2	Coriander	Rural market	17.12.18	37.8 $\pm$ 0.8	2/2
3	Coriander	Rural market	13.01.19	37.3 $\pm$ 0.2	2/2
4	Coriander	Wholesale market	15.01.19	35.0 $\pm$ 0.5	2/2
5	Coriander	Supermarket	27.02.19	35.1 $\pm$ 0.7	2/2
6	Coriander	Rural market	27.02.19	37.2 $\pm$ 0.7	2/2
7	Coriander	Wholesale market	27.02.19	38.8 $\pm$ 0.1	2/4***
8	Coriander	Rural market	17.03.19	39.2 $\pm$ 0.3	3/4***
9	Coriander	Wholesale market	19.03.19	35.9 $\pm$ 0.4	2/2
10	Coriander	Supermarket	19.03.19	35.1 $\pm$ 0.4	2/2
11	Coriander	Rural market	12.04.19	37.8 $\pm$ 0.0	2/2
12	Coriander	Wholesale market	16.10.19	34.9 $\pm$ 0.2	2/2
13	Coriander	Supermarket	16.10.19	36.1 $\pm$ 0.5	2/2
14	Coriander	Rural market	19.10.19	37.6 $\pm$ 0.4	2/2
15	Lettuce	Supermarket	16.04.18	36.8 $\pm$ 0.5	2/4
16	Lettuce	Rural market	20.04.18	37.4 $\pm$ 0.9	2/2
17	Lettuce	Supermarket	04.05.18	36.9 $\pm$ 0.5	2/2
18	Lettuce	Rural market	04.05.18	38.9 $\pm$ 0.6	2/4
19	Lettuce	Wholesale market	04.06.18	37.0 $\pm$ 0.6	2/4
20	Lettuce	Wholesale market	09.07.18	38.5 $\pm$ 0.0	2/2
21	Lettuce	Rural market	13.07.18	35.9 $\pm$ 0.1	2/2
22	Lettuce	Wholesale market	21.09.18	31.8 $\pm$ 0.0	2/2
23	Lettuce	Rural market	21.09.18	36.6 $\pm$ 0.1	2/2
24	Lettuce	Wholesale market	17.12.18	38.8 $\pm$ 0.1	3/4
25	Lettuce	Rural market	17.12.18	37.8 $\pm$ 0.4	3/4
26	Lettuce	Rural market	13.01.19	36.8 $\pm$ 0.4	2/2
27	Lettuce	Supermarket	15.01.19	37.4 $\pm$ 0.4	3/4
28	Lettuce	Supermarket	27.02.19	33.9 $\pm$ 0.1	2/2
29	Lettuce	Rural market	27.02.19	37.2 $\pm$ 1.1	2/2
30	Lettuce	Wholesale market	27.02.19	39.1 $\pm$ 0.2	2/4
31	Lettuce	Rural market	17.03.19	38.3 $\pm$ 0.9	2/2
32	Lettuce	Wholesale market	17.04.19	38.3 $\pm$ 0.6	2/2
33	Lettuce	Rural market	09.08.19	35.0 $\pm$ 0.1	2/2
34	Lettuce	Wholesale market	16.10.19	37.0 $\pm$ 0.5	2/4
35	Lettuce	Supermarket	16.10.19	37.4 $\pm$ 0.3	2/2
36	Lettuce	Rural market	19.10.19	37.2 $\pm$ 0.0	2/2
37	Parsley	Rural market	12.10.18	34.8 $\pm$ 1.4	3/4
38	Parsley	Supermarket	15.10.18	37.8 $\pm$ 0.6	2/2
39	Parsley	Wholesale market	15.10.18	38.3 $\pm$ 0.6	2/4
40	Parsley	Rural market	27.02.19	38.1 $\pm$ 0.7	2/2
41	Parsley	Wholesale market	27.02.19	36.1 $\pm$ 0.1	2/2

42	Parsley	Rural market	17.03.19	38.3 ± 0.8	3/4
43	Parsley	Rural market	12.04.19	39.3 ± 0.7	2/4
44	Parsley	Wholesale market	16.10.19	35.4 ± 1.4	3/4
45	Parsley	Supermarket	16.10.19	37.5 ± 0.9	2/2
<i>Giardia duodenalis</i>					
Sample number	Product	Origin	Collection date	Cqm ± σ	Number of positive wells/ Total
46	Coriander	Rural market	21.09.18	33.1 ± 0.4	2/2
47	Coriander	Wholesale market	16.05.19	37.0 ± 0.4	2/2
48	Coriander	Rural market	09.08.19	38.3 ± 1.6	2/2
49	Parsley	Supermarket	17.09.19	39.8 ± 0.2	2/2

* Positive sample: Cqm value < 40. ** Positive sample for both wells during the first qPCR.

*** sample positive for a single well during the first qPCR, and subjected to a second qPCR where at least 2/4 wells are positive.

Figures

Figure 1

Prevalence of *T. gondii* and *G. duodenalis* in leafy greens Number of positive vegetable samples (mean Cq < 40) for *T. gondii* and *G. duodenalis* by qPCR, collected from different markets in Marrakech, between April 2018 and October 2019