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Checking the detail in retail: Occurrence of *Cryptosporidium* and *Giardia* on vegetables sold across different counters in Chandigarh, India

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ABSTRACT

Fresh produce has been recognized as a vehicle of infection for protozoan parasites, particularly *Cryptosporidium*, and, to a lesser extent, *Giardia*. For both parasites, outbreaks associated with fresh produce have been documented. Although documented outbreaks tend to be from industrialized countries, contamination of fresh produce with these parasites is a global issue. In developing countries, infections with these parasites are often endemic in the community, and basic infrastructure and hygiene measures may be inadequate, thus the likelihood of contamination of fresh produce with these parasites may be higher. Realization of the importance of this transmission route comes against a backdrop of raw salads and more Western culinary habits gaining a foothold, and fresh produce being encouraged as part of the diet due to their associated health benefits. However, if consumption of uncooked fresh produce is going to increase its market sector in India, it is important that it is safe. In this study, various types of fresh produce obtained from three types of vendors in Chandigarh, a major city in Northern India, were analyzed for contamination with *Cryptosporidium* oocysts and *Giardia* cysts using a method that has been previously validated in inter-laboratory spiking experiments. A total of 284 samples of different fresh produce items were analyzed, obtained from the different retailers situated in different societal layers of the city. The overall prevalence of contamination of fresh produce with these parasites was just under 11%, with 6% of the vegetables contaminated with *Cryptosporidium* oocysts, and 5% with *Giardia* cysts. Contaminated vegetables included turnip, cabbage, carrot, chili, coriander, cucumber, radishes, and tomatoes. Molecular analyses identified contamination with *Cryptosporidium parvum* and *Giardia duodenalis* of Assemblage A and Assemblage D, indicating that contamination from animals may be of relevance. Although the prevalence of contamination is similar to those reported in previous studies, the levels of contamination on some items of fresh produce were relatively high. Although the different socioeconomic areas of Chandigarh from which the samples were obtained was not associated with likelihood of contamination, fresh produce from supermarkets had heavier contamination with *Cryptosporidium* oocysts than fresh produce purchased through other sales outlets. The results are discussed in relation to the fresh produce chain and sales models in Chandigarh, both in terms of where contamination may occur and the potential importance of fresh produce as a transmission vehicle.

1. Introduction

Cryptosporidium spp. and *Giardia duodenalis* are among the most frequently occurring intestinal protozoan parasites in humans and animals worldwide (Fayer, 2004; Thompson and Monis, 2004; Thompson, 2004). Both parasites can cause diarrheal disease. Global studies have revealed that *G. duodenalis* and *Cryptosporidium* spp. are two of the most common etiological agents in pediatric diarrhea in developing countries, and are associated with mortality as well as morbidity (Kotloff et al., 2013; Platts-Mills et al., 2015).

The biology of *Cryptosporidium* and *Giardia* makes them suitable for transmission via fresh produce; they have a low infectious dose, a robust transmission stage, are small sized, and some genotypes have a zoonotic potential, giving the opportunity for contamination to occur

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from both animal and human sources (Robertson and Lim, 2011; Robertson and Fayer, 2012). Infected individuals also have a high excretion rate, ranging from $> 5 \times 10^3$ to 9.2×10^5 oocysts per gram feces for *Cryptosporidium* (Goodgame et al., 1993) and 580,000 cysts per gram feces may be shed over a period of days or longer in the case of *Giardia* infection (Danciger and Lopez, 1975).

As fewer people are affected in a foodborne outbreak than in a waterborne outbreak, and may be more scattered geographically (see, for example, the UK outbreak described by McKerr et al. (2015)), a lack of prompt diagnosis may hamper epidemiological investigation. It is worth noting that the last major waterborne outbreak of giardiasis in Europe, in which over 1500 people were infected, took several weeks to be recognized as a waterborne outbreak (Robertson et al., 2006), and in the UK, the specific produce causing the outbreak was never detected.

Cryptosporidium and *Giardia* can contaminate food as a surface contaminant. Contamination with small numbers of infectious (oo)cysts in produce that receives minimal washing or treatment prior to ingestion, poses a threat to public health. Food products can be contaminated directly by cysts and oocysts in feces from humans and animals or via the environment, such as soil and water, and thus serve as vehicles for transmission, at any step in the farm-to-fork chain. For fresh produce, contamination may persist until infection as the production chain requires cool temperatures and moist conditions to maximize food freshness, and that also enhance survival of *Giardia* cysts; *Cryptosporidium* oocysts seem to be more tolerant to temperature changes on fresh produce (Utaaker et al., 2017). For foods such as, for example, bakery produce, storage conditions (dry, at room temperature) are likely to be deleterious to parasite survival. Although there has been discussion around *Cryptosporidium* oocysts surviving for longer in conditions of cool temperatures and high humidity, it has nevertheless been concluded that the likelihood of foodborne outbreaks occurring is no greater in cooler environments than anywhere else in the world (Robertson and Chalmers, 2013). Indeed, the foodborne transmission route is probably particularly relevant in places where infection is more likely underdiagnosed and underreported, and especially so in the developing countries where infrastructure and resources for investigation and reporting are limited. However, in such settings, where various intestinal infections are endemic, outbreaks caused by contamination of food or water may be more difficult to identify against a background of high infection.

Methods for detecting contamination of foodstuff by protozoans had been relatively poorly developed until recently, until the publication of ISO Method 18744 (ISO, 2016). However, this method is both expensive and time-consuming, and essential reagents must be stored refrigerated. To implement such methods for routine analysis in laboratories that are already poor in resources may be prohibitively expensive and impractical. Furthermore, considering the vast amount of fresh produce from different traders in the chain of retail events in a developing country's retail model, using lab analyses may provide scant information regarding tracking the sources of contamination. Nonetheless, these methods enable surveys to be conducted and an assessment of contamination levels to be made, and such data are essential for assessment of risk and determining the extent of significance of such contamination.

In this study, the aim was to analyze fresh produce sold at different retailers in Chandigarh, India for the occurrence of *Cryptosporidium* oocysts and *Giardia* cysts, and to use molecular typing of parasites from positive samples as a further indication of the possible sources of contamination.

2. Materials and methods

2.1. Sampling site

Over a two-year period between February 2014 and February 2016, 284 vegetables were purchased at local mandi, street vendors, and supermarkets in Chandigarh, Northern India.

Chandigarh is a union territory of its two neighboring states, Haryana and Punjab, although not considered a part of either state. According to the State Agricultural Marketing Board of the Union Territory Chandigarh, Chandigarh has no major crop itself, and most fresh produce available in Chandigarh comes from these neighboring states. The Union Territory Chandigarh has only a limited area available for agriculture, and this land is being gradually diminishing with the expansion of Chandigarh city. In addition, farmers who keep a large number of dairy cattle utilize these areas to grow fodder for livestock (Chandigarh Administration, 2016a).

Chandigarh has only one principal Market Yard, and there are no official seasonal or other kinds of market yards, or any unregulated markets (C.S.A.B, 1961) Thus, most of the samples collected in this study came via the principal Market Yard, from where they are distributed to different trading hubs and retailers.

Chandigarh is a city undergoing rapid growth and development. The city is organized according to “phases”, which can be a proxy for socio-economic status, as reflected by density of inhabitants: Phase I (higher socio-economic status), Phase II (moderate socio-economic status), Phase III and non-sectorial villages (lower socio-economic status). In addition, Chandigarh is divided into sectors, based on the grid concept of the roads, and different sectors are also considered more or less affluent than others, according to the Chandigarh Master Plan – 2031 (Chandigarh Administration, 2016b).

2.2. Source of samples of fresh produce

The sampling strategy aimed at analyzing vegetables representing each sector of the city, including both rural and urban areas, the “phases” of the city, and to enable comparison of the three main sales types used for fresh produce in India: street vendors, mandi (see description below), and supermarkets. Nevertheless, due to access to vegetables and markets, the number of samples from each sector are non-uniform, as are the distribution of vegetable types and salespoints (see Table 1).

Among the total of 284 samples, 137 (48%) were obtained from individual street vendors, with either a stationary or mobile salespoint. The vendor handles the produce until it is purchased by the customer. In addition, 125 (44%) samples were obtained at mandi, which are local trading hubs for agricultural produce, and are arranged on a regular weekday basis in different sectors in Chandigarh. At the mandi, the local producer or salesperson brings his products and displays them for the customer. As with street vendors, only the vendor handles the foodstuffs until purchased by the customer.

The remaining 22 (8%) samples were obtained from modern supermarkets, where the vegetables are displayed for the customer to choose their preferred items, touching and handling the produce as they make their selection before paying at the counter.

The samples were collected from different areas (phases), or sectors, of Chandigarh, thus also representing vegetables from different socio-economic layers, as well as salespoints. Of the 284 samples, 119 were collected from Phase I sectors, 112 were collected from Phase II sectors, and 51 were collected from sectors in Phase III and non-sectorial villages.

The samples most probably all came from the same principal Market Yard, but were sold in different areas of the city, and thus under different conditions.

2.3. Fresh produce selected for analysis and their use in India

The vegetables to be analyzed were chosen according to the season of availability, with emphasis on those commonly consumed raw. These included coriander leaves, lettuce, radish, tomatoes, cucumber, fenugreek leaves, cabbage, chili, mint leaves, carrot, and turnip.