

## Pathogens in crop production systems irrigated with low-quality water in Bolivia

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### ABSTRACT

In dry areas, the need for irrigation to ensure agricultural production determines the use of all available water sources. However, the water sources used for irrigation are often contaminated by untreated or minimally treated wastewater. Microbial risks from reusing wastewater for vegetable irrigation can be addressed by installing environmental barriers that pathogens must cross to reach humans in the reuse system. Knowledge of pathogen flows inside the system and pathogen removal potential is the first step towards devising a risk management strategy. This study assessed microbe prevalence in farming systems in the Bolivian highlands that use wastewater-polluted sources for irrigation of lettuce. Samples of soil, lettuce and different water sources used in the farming systems were taken during one crop season and concentrations of coliphages, *Escherichia coli* and helminth eggs were measured. The results showed high spread of these microorganisms throughout the whole system. There was a significant correlation between microbial quality of water and of the harvested produce for several microorganisms. The microbial prevalence in protected shallow wells was found to be significantly lower than in other water sources. These findings can help formulate feasible risk management strategies in contexts where conventional technologies for microbial removal are not possible.

**Key words** | hygiene, nutrient recycling, riverbank filtration, vegetables, wastewater

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### INTRODUCTION

The increase in food production worldwide in recent decades has been made possible by increasing use of mineral fertilisers and water. However, sustainable supply of both is not assured in the long run due to depletion, uneven distribution and climate change (Childers *et al.* 2011; Malik *et al.* 2015). Therefore, sustainable solutions to such future challenges will likely include closing the loops in the

human cycle of nutrients and water (Scott *et al.* 2015). Manure and wastewater are major sources of nutrients that can be reused in agriculture, potentially reducing the pressure on production of chemical fertilisers and the eutrophication risks from their discharge to sensitive aquatic systems (Scott *et al.* 2004; Sommer 2013). In addition, the reuse of wastewater reduces the pressure on other water sources (Keuckelaere *et al.* 2015), which is especially important in arid and semi-arid areas (Scott *et al.* 2004). However, closing the loop of nutrients and water poses hazards for human and environmental health and food safety, due to the risk of pathogens and toxic compounds being introduced

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into agricultural production systems unless properly managed (Scott *et al.* 2004; Randolph *et al.* 2007; Sommer 2013). The situation is particularly problematic in low-income countries, where wastewater is poorly treated or not treated at all (Scott *et al.* 2004) and sanitisation of manure is poorly controlled or unregulated (Randolph *et al.* 2007). Thus, the use of wastewater and manure in agricultural systems is desirable in terms of sustainability of food production systems, but also poses threats to public and environmental health which must be properly addressed.

Wastewater irrigation and application of manure are associated with an increased burden of infectious diseases due to food contamination (Keuckelaere *et al.* 2015). Secondary wastewater treatment systems are poor at removing microorganisms, so there is a risk of infection from the high load of pathogens even when wastewater is treated prior to irrigation (Ottozon *et al.* 2006). Infectious diseases can hamper the body's ability to metabolise nutrients, leading to malnutrition or undernutrition (Fanzo 2014). Together, malnutrition, undernutrition and infectious diseases are the greatest risk factors in the global burden of disease, representing 16% of global disability-adjusted life years (DALY) (Lopez *et al.* 2006). If this burden is added to that arising due to unsafe water, sanitation and hygiene (3% global DALY), the total is 19% global DALY (Lopez *et al.* 2006).

The Stockholm framework has been promoted worldwide by the World Health Organization (2006) to address the microbiological risks from reusing wastewater, based on the notion that the effectiveness of pathogens in causing disease depends on both epidemiological characteristics and environmental barriers that they must cross to reach humans. A barrier can be defined as an event or a physical obstruction that prevents transmission, reduces infectivity or decreases pathogen concentrations (Nordin 2007). The risks of disease can be managed by knowing and handling the relevant characteristics of each wastewater reuse system.

In Bolivia, the major infections attributable to water, sanitation and hygiene are diarrheal diseases and intestinal nematodes (1,500 and 104 episodes per 100,000 inhabitants, respectively) (Pruss-Ustun *et al.* 2008). Despite a lack of data directly linking wastewater with such diseases, the use of wastewater for irrigation is a well-known practice and its potential in disease transmission has long been recognised (Huibers *et al.* 2004). At farm level, various strategies for

wastewater reuse in irrigation (e.g. indirect reuse, change of crops, riverbank filtration of polluted streams) have been implemented (Huibers *et al.* 2004; Verbyla *et al.* 2016), but their effect on the microbial quality of produce has not been well studied. Therefore, data on microbial prevalence throughout farming systems are needed in order to begin clarifying the relationship between wastewater irrigation of vegetables, the farm-level irrigation strategy used and the disease burden on the population.

The aim of this study was to assess the pathogen flows throughout a water reuse system for production of lettuce in an agricultural area located in a peri-urban zone of Cochabamba city, Bolivia, in order to identify and evaluate the existing environmental pathogen barriers. For this purpose, viral and bacterial indicators and helminth eggs were quantified in soil, water and lettuce samples taken during one crop season (August–December 2014) and complemented with some data from the next crop season (February–April 2015). The data obtained were then statistically processed and used to analyse: (i) the microbial contamination in the system; and (ii) the likely flows of microbial contamination throughout the system components (i.e. soil, water and produce).

## METHODS

### Characteristics of farming systems in the study area

The study area is within a region of intensive vegetable production known as Huerta Mayu, located at about 2,600 m a.s.l. in intermountain valleys of the Andes in Bolivia. The farming systems examined in detail in the study are located in a 3-hectare sub-area where the soils are predominantly silty and originate from lake and fluvial deposits, and the subsurface layer through which water flows consists of sands, clays and igneous rocks (Metternicht & Fermont 1998; Verbyla *et al.* 2016). Irrigation is necessary for crop production because the climate in the area is semi-arid and most rainfall events are short and intense (mean annual temperature 14–17 °C, mean annual rainfall 400–600 mm but <40 mm between April and September) (Metternicht & Fermont 1998). Irrigation water is obtained through riverbank filtration from a river heavily polluted with domestic

wastewater (the river Rocha) (Contraloría General del Estado 2011). There are no available data regarding the natural water flow in the river, but it is believed that almost all river flow in the dry season consists of domestic and industrial wastewater (Huibers *et al.* 2004).

The crop rotations in the area are characterised by intensive production. The dominant crop between September and April is lettuce, occasionally replaced by radish and much less frequently by other short-cycle plants (e.g. chard, beetroot). During winter, more low temperature-resistant crops are grown. The dominant crop in this season is onion, occasionally replaced by potato. Lettuce and radish crops have a short cycle (less than two months after the nursery), so are planted several times a year. Thus, the main crop in terms of number of cultures per year in Huerta Mayu is lettuce.

Irrigation is carried on in the dry season (March–November), and is applied through furrows, two or three times a week, at either 7–11 a.m. or 4–8 p.m. Irrigation management depends on several factors, such as pump capacity, the nature of the crop, the occurrence of rainfall and arrangements among farmers (i.e. water use rights). The water is mainly pumped from riverbank filtration (RBF) extraction wells. In this study, two types of RBF wells were identified in the area: unprotected and protected. Unprotected wells have a diameter greater than 5 m at the wellhead and have no protection (i.e. lining or cap) against external factors such as animal waste, vegetation or surface runoff. Protected wells have a diameter of ~1 m, their walls are lined with concrete rings surrounded by a thin layer of sand and the top is protected with a lid which is removed and replaced for every irrigation event. All the wells have been manually dug to a depth of 5–6 m. Although each RBF well has its own assigned plot, factors such as low water levels can force the farmers to use other water sources, e.g. other wells or water direct from the river. Although not reported by farmers, signs of irrigation using such alternative sources (another well or river) were found at least twice in experimental plots during the study, so it was assumed that this could have happened on more occasions without being detected.

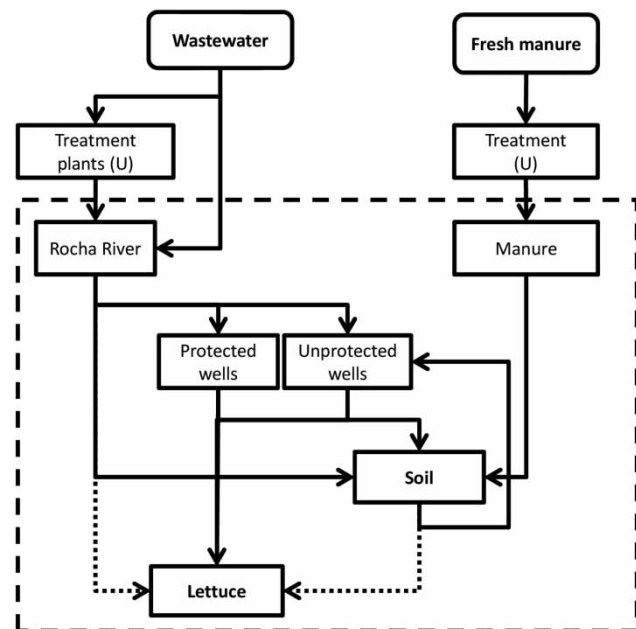
Fertiliser is applied twice during the lettuce growing season. On the first occasion, urea and cattle manure are applied during plot preparation before transplanting. The

second application is performed one month after transplanting and only urea is used. The manure comes from different local farms and its level of treatment is uncertain.

There are two main sources of pathogens in agricultural systems in Huerta Mayu: contaminated irrigation water and contaminated manure. The flow of pathogens from human and animal excreta until contact with the product is illustrated in Figure 1.

### Microbial prevalence in farming systems

Data were obtained by sampling and analysing water from the river, three unprotected wells (located at 16, 25 and 42 m from the river), two protected wells (located at 19 and 80 m from the river), five field plots served by these wells and lettuce samples from the selected field plots during the crop culture prior to the rainy season (October–December 2014). All farming operations were carried out by farmers. River water was sampled to obtain an approximate value of how much microbial pollution could be reduced by RBF or added to the farming system through occasional irrigation using river water. Additional samples



**Figure 1** | Pathogen flows in the water reuse system in the study area of Huerta Mayu, Bolivia. Solid arrows indicate likely microbial flows, while dotted arrows show possible microbial flows. The part of the diagram inside the dotted square represents the agricultural system in Huerta Mayu. (U) indicates that actual levels of treatment are uncertain.

were taken in March–May 2015 for determination of some parameters (according to available laboratory capacity), in order to maximise the representativeness of the data. The total number of samples analysed and the time of collection are presented in Table 1.

All water samples were collected between 9 and 11 a.m., during the period in which some farmers irrigate. Sterilised plastic 200-mL bottles were used for coliphages and *E. coli* sampling, while 10-L plastic containers were used for helminth samples. Well water was collected using a metal bucket and transferred to bottles and containers. The metal bucket was dried, flamed and allowed to cool between sampling points, in order to avoid cross-contamination. Once closed, the bottles were placed in plastic bags on ice and immediately transported (i.e. 2 hours or less) to the laboratory, where they were stored in a cold chamber at 4 °C until analysis. Both *E. coli* and coliphages samples were processed on the day of collection. Processing of samples for helminth eggs was performed between 1 and 4 weeks after sampling, according to the capacity of the laboratory.

Lettuce sampling was carried out at the end of the last cropping season prior to the rainy season (i.e. November and December). In each plot, individual leaves of randomly selected plants were collected until three composite samples of about 500 g each were obtained. All composite samples were placed in sealed plastic bags and transported to the laboratory, where they were analysed for *E. coli* and coliphages and then stored at 4 °C until helminth analysis.

Soil samples were collected from the top 7 cm at points determined through the intersections of a grid formed by squares with 4–6 m sides in every field plot. All samples of the same plot were mixed, to give one composite sample of about 2 kg. These composite samples were placed in plastic bags and transported to the laboratory, where they were analysed on the same day for *E. coli* and coliphages and then stored at 4 °C until helminth analysis.

### Microbial analysis

The analytical methods used for analysis are presented in Table 1. For analyses on solid samples (i.e. lettuce and soils), the same procedures as used for water samples were followed, but complemented with the Tulane method for helminth analysis and following the procedure described by Verbyla *et al.* (2016) for *E. coli* and coliphages. The detection limit of the analytical methods used was: 2.6 eggs L<sup>-1</sup> for helminths in water, 0.5 and 0.1 eggs g<sup>-1</sup> for helminths in soil and on lettuce, respectively, 20 colony-forming units (cfu) L<sup>-1</sup> for *E. coli* in water, 1,000 cfu g<sup>-1</sup> for *E. coli* in soil and on lettuce, 10 plaque-forming units (pfu) L<sup>-1</sup> for coliphages in water, and 0.2 and 0.1 pfu g<sup>-1</sup> for coliphages in soil and on lettuce, respectively.

### Data analysis

In order to obtain an approximation of the level of microbial contamination in the system, the data obtained were compared against the threshold values for irrigation water from

**Table 1** | Number of samples collected from the river, protected and unprotected wells, soils and lettuce, time span of collection and the corresponding analytical method used for determination of microbe content

	Helminth eggs				<i>Escherichia coli</i>		Coliphages (F <sup>+</sup> specific and somatic coliphages)			
	Oct-Dec		Mar-May		Oct-Dec		Oct-Dec		Mar-May	
River	2	a	3	a	4	b	2	c	3	c
Unprotected wells	17	a	9	a	14	b	13	c	12	c
Protected wells	12	a	6	a	8	b	8	c	8	c
Soil before planting	5	a*	–	–	5	b*	5	c*	–	–
Soil at harvest	5	a*	–	–	5	b*	5	c*	–	–
Lettuce	11	a*	–	–	4	b*	4	c*	9	c*

\*Mexican norm AA-113-SCFI-2012; \*complemented with Tulane method (Bowman *et al.* 2003); <sup>b</sup>EPA 1603 method; <sup>b\*</sup>modified according to Verbyla *et al.* (2016); <sup>c</sup>modified EPA 1602 method; <sup>c\*</sup>modified according to Verbyla *et al.* (2016).

several national regulations in the case of water and against the US-EPA classification of biosolids from 2002 in the case of soil. Values below the detection limit were estimated by robust-order statistics assuming log-normal distribution, and the data were used to generate boxplots. In order to assess the flows of fecal microorganisms in the farming systems of Huerta Mayu, two types of statistical tests were carried out: (i) logarithmic regressions (Kadlec & Wallace 2008) were applied to correlate the distance of RBF wells from the river and the microbiological quality of RBF wells; and (ii) Mann–Whitney contrasts at 95 and 99% confidence level were performed to compare: (a) the microbiological quality of lettuce irrigated with water from protected wells and water from unprotected wells; (b) the microbiological quality of soil irrigated with water from unprotected wells and protected wells; and (c) the microbiological quality of soil at sowing and at harvest. The microbiological quality of water sources (i.e. unprotected and protected wells) was also compared at 95 and 99% confidence level by Mann–Whitney contrasts (when datasets for contrasts had less than 40 samples) or by t-test for two samples (when datasets for contrasts had 40 samples or more).

## RESULTS

### Microbial concentrations

The microbial concentrations in water from RBF wells displayed an inverse relationship with distance from the river. It was also found that microbial concentrations in water from unprotected wells were higher than those in water from protected wells (Figure 2(a)). The median concentrations of helminth eggs, *E. coli* and coliphages detected in water followed the trend: River > unprotected wells > protected wells (Figure 2(b)). In addition, concentrations below the detection limit (BDL) were only found in water from protected wells (the percentage of protected well water samples below the detection limit was: 17% *Taenia* spp., 44% *Trichuris* spp., 12% *E. coli* and 19% coliphages).

The median concentrations of microbes per gram of soil found before planting were 6.1 *Ascaris* spp. eggs, two *Taenia* spp. eggs, two *Trichuris* spp. eggs,  $1.1 \times 10^3$  *E. coli* cfu and 46 coliphages pfu (Figure 3). The median concentrations per

gram of soil found after harvest were slightly higher: 7.5 *Ascaris* eggs, 5.4 *Taenia* spp. eggs, 4.4 *Trichuris* spp. eggs,  $4.3 \times 10^3$  *E. coli* cfu and 66 coliphages pfu. The concentrations in all soil samples were above the detection limit.

Helminth eggs were detected in all lettuce samples analysed. The median concentrations per gram were one *Ascaris* spp. egg, 0.5 *Taenia* spp. egg (8% samples BDL),  $1.9 \times 10^3$  *E. coli* cfu and 5.1 coliphages pfu. The *Trichuris* spp. egg median concentration was 0, but this is because 54% of the samples had values below the detection limit (i.e.  $<0.1$  eggs  $g^{-1}$ ). The average *Trichuris* spp. concentration in lettuce samples was 0.1 eggs  $g^{-1}$ .

### Flows of fecal microorganisms in the farming systems

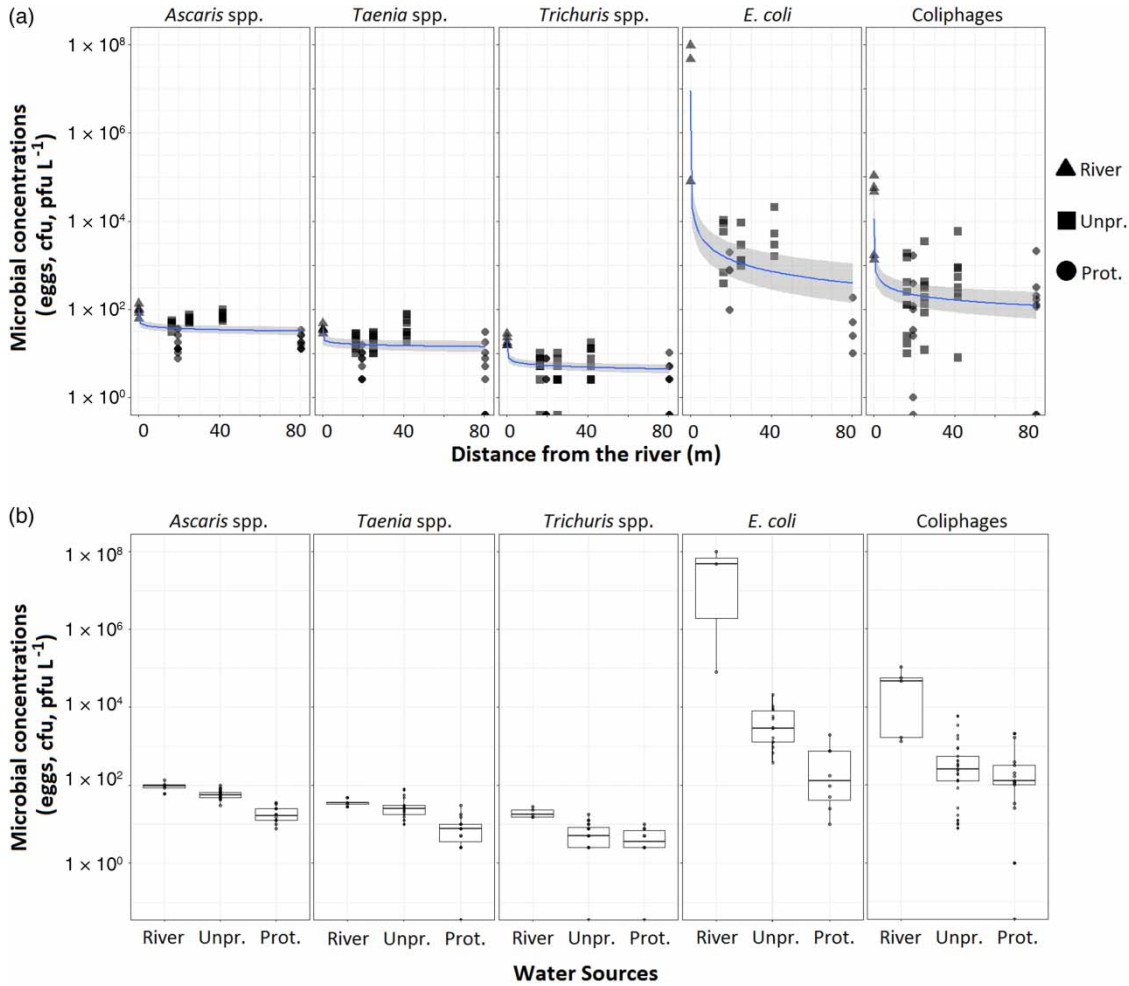
The inverse relationship between distance from the river to the RBF wells and their microbial concentrations was found to be significant for most organisms investigated, explaining about 50% of the variance in *E. coli* and coliphages (Table 2). The concentrations of all microbes except coliphages were significantly higher in water from unprotected wells than in water from protected wells (Table 2). Despite a few significant differences in microbial concentrations, no clear pattern was identified with any statistical test performed on soil data (Table 2). On comparing the two major water sources (protected and unprotected wells), significantly higher concentrations of *Ascaris* spp. and *Taenia* spp. were found on lettuce irrigated with water from unprotected wells (Table 2). There was no significant difference in coliphage concentrations on lettuce irrigated with water from the two well types. A similar comparison was not possible for the other two organisms investigated, due to the limited number of samples (Table 1).

## DISCUSSION

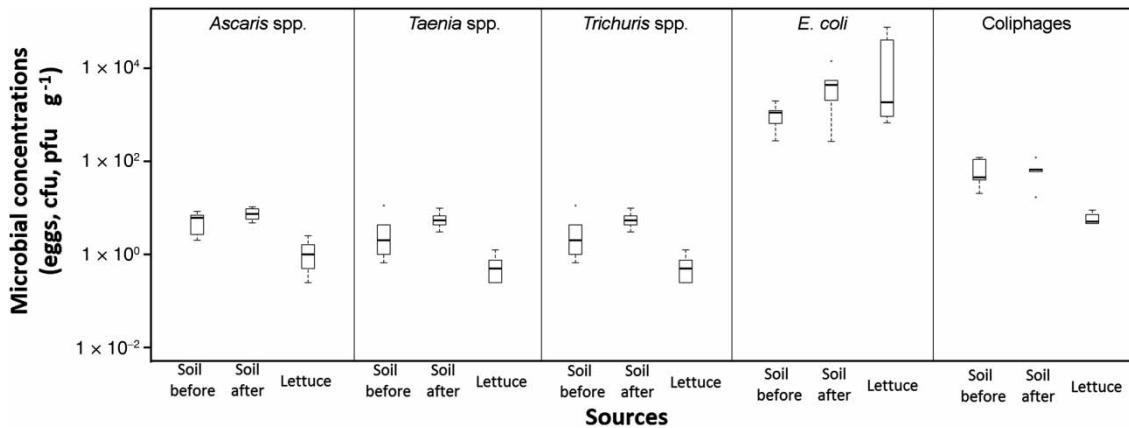
### Microbial contamination in Huerta Mayu farming systems

Concentrations of the studied microbes in Huerta Mayu farming systems were similar to or higher than threshold values stated in regulations and in published studies on similar systems. The concentrations of *E. coli* and helminth eggs





**Figure 2** | Graphical representation of the occurrence of the different microorganisms studied, (a) according to distance to the river and water source and (b) for the three types of water sources investigated: river, unprotected wells and protected wells. The logarithmic regressions performed to assess the influence of distance from soil on microbial concentrations in the well are also shown in (a).



**Figure 3** | Graphical representation of the occurrence (eggs, cfu, pfu g<sup>-1</sup>) of the different microorganisms studied, shown separately for soil prior to sowing, soil after harvest and harvested lettuce.

**Table 2** | Results of statistical comparisons and logarithmic regressions performed on the parameters analysed, when number of samples was not sufficient to carry out a comparison, it is denoted not applicable (n.a.)

	Comparison 1		Comparison 2		Comparison 3		Comparison 4		Log. regression	
	Lettuce with unprotected wells (a)	Lettuce with protected wells (b)	Soil with unprotected wells (c)	Soil with protected wells (d)	Soil at sowing (e)	Soil at harvest (f)	Water from unprotected wells (g)	Water from protected wells (h)	R <sup>2</sup> of model for microbial concentrations (y) in water at variable distances from the river y = a + b·ln(distance)	
<i>Ascaris</i> spp.	a > b**		c > d**		e = f		g > h**		0.32**	
<i>Taenia</i> spp.	a > b**		c = d		e = f		g > h**		0.08	
<i>Trichuris</i> spp.	n.a.		c = d		e < f*		g > h**		0.50**	
<i>E. coli</i>	n.a.		c = d		e = f		g > h**		0.52**	
Coliphages	a = b		c = d		e = f		g = h		0.46**	

> significantly more than; = not significantly different from; < significantly less than.  
\*\*α = 0.01; \*α = 0.05.

found in samples from the three water sources (Figure 2) exceeded levels set by several national regulations for irrigation of leaf crops (i.e. 10<sup>3</sup> *E. coli* L<sup>-1</sup> and >1 nematode egg L<sup>-1</sup>) according to a review by Uyttendaele et al. (2015). Regarding soil samples, the microbial concentrations detected (Figure 3) correspond to US-EPA class B biosolids (i.e. >10<sup>6</sup> thermotolerant coliforms CFU gr<sup>-1</sup> and the presence of *E. coli*, enteroviruses and parasites), which means that soil microbial quality is similar to that of treated sewage sludge and its use for leaf vegetable cropping would not be permitted (Santamaria & Toranzos 2003). The levels of microorganisms found on lettuce (median 5.1 coliphages g<sup>-1</sup>, 1.9 × 10<sup>3</sup> *E. coli* cfu g<sup>-1</sup> and 0.1–1 helminth eggs g<sup>-1</sup>; Figure 3) were similar to those reported in other studies on irrigation of lettuce and other leaf vegetables with wastewater, e.g. 10 coliphages pfu g<sup>-1</sup> lettuce (Song et al. 2006), 1.3 × 10<sup>4</sup> *E. coli* pfu g<sup>-1</sup> cabbage (Mhongole et al. 2016) and 1.1 helminth eggs g<sup>-1</sup> leafy vegetables (Ensink et al. 2007). The prevalence of *Ascaris* spp. and *Trichuris* spp. eggs (100 and 46%, respectively) was higher than previously reported, e.g. Gupta et al. (2009) reported a prevalence of 36% for *Ascaris* spp. and 2% for *Trichuris* spp. on wastewater-irrigated lettuce. Therefore, it can be stated that the levels of microbial contamination in the farming systems in Huerta Mayu are high despite the on-farm strategies implemented by farmers.

### Flows of fecal microorganisms in water sources for irrigation

As mentioned, manure and wastewater are the main sources of pathogens in the Rocha river basin (Figure 1). However, the basin has no intensive animal farming, so cow and chicken manure are brought in for fertilisation. Most farmers buy manure from dairy farms that feed cattle with forage crops, irrigated with water from the same river (Rocha) and with effluent from the largest wastewater treatment plant in Cochabamba city (Huibers et al. 2004), making manure a potential source of *Taenia* spp. (Feachem et al. 1983). Moreover, *E. coli* and coliphages can both be present in cow and chicken manure. Besides manure, wastewater is considered to be the other relevant source of pathogens in the study region (Figure 1). The majority of the wastewater generated in the Rocha basin is discharged

into the river from many sewage systems after no or poor treatment (Contraloría General del Estado 2011). Whatever their origin, once pathogens are released into water bodies or soil, their fate depends on numerous environmental interactions, with water content and fluxes (e.g. runoff, flow through porous media) being crucial.

The water in the RBF wells was found to be of higher quality than river water, exhibiting decreasing microbial concentrations with increasing distance from the river, but with higher microbial concentrations in unprotected than in protected wells (Figure 2, Table 2). The origin of pathogens in the wells could be surface runoff through the wellhead and river water intrusion through the soil layers (i.e. riverbank filtration) (Santamaria & Toranzos 2003; Verbyla *et al.* 2016). Increasing microbe removal rate with increasing water travel time (distance from the river) has been widely reported in previous studies with RBF systems and is linked to increasing opportunities for contact between microbes and the filtration medium (Sprenger *et al.* 2011). The fact that about 50% of the variance in bacterial and viral concentrations in the RBF wells was explained by distance from the river (Table 2) highlights the relevance of microbial flow through the riverbank soil. Following the same reasoning, the lower percentage of variance in helminth egg concentrations in RBF water explained by distance from the river (Table 2) reveals the importance of microbial flow through surface runoff. The size of helminth eggs limits their movement through the soil, so when they are detected in water it is often because of contaminated surface water flowing directly into groundwater, either through the wellhead or through pathways of sufficient size in the soil layers (Feachem *et al.* 1983). Thus, it seems that the efficiency of RBF wells in improving the microbial quality of water is counteracted by surface runoff of water to these wells.

The higher microbial concentrations in unprotected wells (Table 2) indicated a significant positive effect of lining and lidding wells on the microbial quality of water. The lid on protected wells probably limits contamination through the wellhead by establishing a waterproof barrier against surface runoff. As regards water intrusion, filtration of microorganisms in the vadose zone depends on several soil properties (e.g. adsorption, pore size, moisture content and temperature) (Powelson & Gerba 1995), some of

which are improved by the layer of gravel/sand and concrete lining in protected wells. A 1 log unit lower *E. coli* median concentration in water from protected wells compared with unprotected wells would reduce the infection risk from lettuce consumption by at least 1 log unit per person per year, according to simulations reported by World Health Organization (2006). Therefore, well protection can act as an additional effective barrier to both forms of microbial flow.

### Dynamics of fecal microorganisms in soil

The likely sources of fecal pathogens in soils in the study area are manure and irrigation (waste)water (Figure 1). However, our results showed no significant difference in the levels of microbial parameters in soil irrigated with water from protected and unprotected wells, or on comparing the same soil after one crop season (Table 2). A major reason for this can be the long-term exposure of soil to both contaminant sources. Addition of manure and well water to soil on a regular basis, combined with use of lower-quality water (i.e. river water or water from other non-assigned wells) when well water is scarce, would have continuously supplied organic matter, humidity and fecal microorganism loads to the soil (Figure 2). Organic matter and humidity are major factors in increased survival of microorganisms in soil (Santamaria & Toranzos 2003). Under such favourable conditions, microorganisms can survive for a longer time and accumulate in soil (e.g. *Salmonella* spp. three months, *Ascaris* spp. 12 months) (Feachem *et al.* 1983). This can lead to the presence of high, stable populations in soil, resulting in similar microbial concentrations in soils regardless of the irrigation water source.

These slow-accumulation dynamics may also explain the slight increase in microbial concentrations in soil after one cropping season (Figure 3). Although this trend was not statistically significant, it suggests that the inactivation rate was lower than the addition rate to this soil, which may result in significant increases over several cropping seasons. This agrees with findings by Hidri *et al.* (2014) that continuously increasing bacterial concentrations in wastewater-irrigated soils can be statistically significant after only one year of irrigation. Consequently, soils would act as a reservoir of



pathogens in the lettuce production system examined in this study.

### Flows of fecal microorganisms to lettuce

Water and soil can both be sources of microbial contamination of produce. As regards water sources, the loads of *Ascaris* spp. and *Taenia* spp. observed on lettuce irrigated with water from unprotected wells were significantly higher than those on lettuce irrigated with water from protected wells (Table 2). On the other hand, no difference was found for coliphages on lettuce irrigated with these two types of waters. Given the agreement observed between microbial quality of lettuce and water for *Ascaris* spp., *Taenia* spp. and coliphages (it was not possible to perform similar comparisons for *Trichuris* spp. and *E. coli*), these results can be taken to indicate a correlation between microbial quality of lettuce and microbial quality of irrigation water. This is in agreement with a number of studies reviewed by Keuckelaere *et al.* (2015), in which different microbial qualities of irrigation water led to different probabilities of crop contamination, indicating the importance of microbial flow from water to produce.

Although microbial flow from soil to produce is possible (Figure 1), the levels of fecal pathogens in soil in the present study did not show any direct influence on pathogen concentrations on produce (Table 2). It is widely accepted that transfer of pathogens from soil to produce occurs mainly by soil splashing during heavy rainfall events (World Health Organization 2006). Soil splashing produced by sprinkler irrigation has recently been shown to be a major mechanism in bacteria transference from soil to leafy vegetables (Allende *et al.* 2017). The absence of rainfall events during the present study and the lack of correlation between microbial concentrations in soil and on lettuce (Table 2) suggest that the flow of pathogens from soil to produce is not significant under furrow irrigation (or at least not as important as the flow of pathogens from irrigation water to produce), even when there are high pathogen concentrations in soil. This might also explain the finding reported by the World Health Organization (2006) of no nematode eggs on lettuce cultivated in soil containing >1,200 *Ascaris* spp. eggs g<sup>-1</sup> (the irrigation technique was not specified). Lack of published studies about the

topic and small sample size in the present study prevent us drawing the conclusion that furrow irrigation is an effective barrier to microbial flow from soil. Given the importance of this process for health risks (due to the long survival of parasite eggs in soils), further investigations are required.

### CONCLUSIONS

- All water sources tested in this study were contaminated with pathogens, including protected (lined and lidded) wells. The high contamination level in water sources was reflected in the other elements of the farming systems (i.e. contaminated soil and lettuce), although soil contamination could not be directly linked to the corresponding well.
- Riverbank filtration was confirmed as a feasible barrier against pathogen flow, but cross-contamination processes can counteract its effect. Structures for well protection proved to have a significant effect in reducing cross-contamination.
- A correlation between water contamination and microbial load on the final produce was found for *Ascaris* spp., *Taenia* spp. and coliphages, indicating that water was the main contamination source for lettuce.
- Furrow irrigation does not appear to be a major factor in contamination of leafy vegetables. It is important to confirm whether furrow irrigation can act as a barrier to pathogen flow, given the widespread use of flooding techniques in wastewater irrigation.
- Although water was the main contamination source for lettuce, additional studies are required to determine the main input of pathogens to the system. Moreover, since the soil acts as a reservoir of pathogens, it is important to determine whether soil can contaminate other elements in the farming system and the long-term importance of pathogen accumulation in soil.

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