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RESEARCH ARTICLE

FRECUENCY AND SPECIES OF C*AMPYLOBACTER* SPP. IN BROILER AT THREE LEVELS OF THE POULTRY PRODUCTION CHAIN OF COSTA RICA

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ABSTRACT

Campylobacter spp. is one of the main bacteria that cause food borne illness. Many of these cases are caused by *C. jejuni* and *C. coli*, which are transmitted mainly by poultry. A cross-sectional study, conducted between March and July 2015, demonstrates the national frequency of *Campylobacter* spp. and the species *C. jejuni* and *C. coli* in broilers for human consumption using a simultaneous sampling design in three levels of Costa Rican poultry production chain. For this research, 152 samples of cecal content (CC) (87 farms), 104 samples of carcass rinse after chiller (CA) (six processing plants), and 96 carcass rinses from 96 retail stores (RS) were collected. The samples were analyzed by microbiological culture and PCR species specific. The overall frequency of *Campylobacter* spp obtained was 59.37% (209/352, 95% CI 54.24%-64.51%), for *C. jejuni* was 42.59% (95% CI 37.45%-48.26%), for *C. coli* was 3.09% (95% CI 1.21%-5.00%) and 8.64% (95% CI 5.62%-11.77%) for contamination with both species. The frequency of *Campylobacter* spp. in CC was 57.23% (95% CI 49.37%-65.10%), in CA was 61.53% (95% CI 52.19%-70.89%) and 60.42% (95% CI 50.63%-70.20%) for RS. The frequency of *Campylobacter* spp. found in this study is high, and it represents a risk for public health in Costa Rica. Preventive measures for this agent are few and inadequate, thus leading to high levels of contamination.

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INTRODUCTION

According to the European Food Safety Authority (EFSA) (European Food Safety Authority, 2015), Campilobacteriosis continues to be the most common cause of reported zoonosis, with a rise in the amount of confirmed cases in the European Union (EU), and USA since 2008 (CDC 2010, European Food Safety Authority 2015.Macé *et al.,* 2016). Additionally, the occurrence of *Campylobacter* spp continues to be high in broiler meat (European Food Safety Authority, 2015). Many of the reported cases are due to *Campylobacter jejuni*, and *C. coli*, which are both transmitted mostly through poultry (Fischer *et al.,* 2013).*C. jejuni* is the predominant species that causes disease in human beings.

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The signs of disease caused by this agent are: diarrhea (might be bloody), abdominal pain, fever, headache, nausea, and vomiting (Farace and Viñas, 2007, Osali *et al.,* 2012). Guillain-Barré can occur to some patients who have suffered campilobacteriosis. (Leonard *et al.,* 2004, Farace *et al.,* 2012, de Boer *et al.,* 2015). In Costa Rica, 23.42kg of food derived from the poultry chain are consumed annually per capita in Costa Rica (Wright, 2010).The high rate of poultry consumption, combined with inadequate treatment during production, sale, and preparation for consumption implicate a higher risk of infection. Because of this, it's crucial to know the current situation of *Campylobacter* spp in the poultry production chain of Costa Rica. This subject is of high importance nationally, and globally, as the poultry chain in Costa Rica has a high economic relevance in local sales, and in exportation. In the past, studies conducted in Costa Rica (Pacheco and Peña 1996, Rojas *et al.,* 1996, Castrillo 2010)

have shown mixed results which contradict each other, possibly due to the use of different culture methodologies, and the identification of different bacteria. A more recent study was conducted to analyze three levels of the poultry production chain simultaneously butwith a small number of samples (Zumbado *et al.,* 2014). The objective of this study was to determine the frequency of thermo tolerant *Campylobacter* spp. and the species *C. jejuni,* and *C. coli* in Costa Rica by simultaneously analyzing three levels of the broiler production chain for human consumption (farms, processing plants, and points of sale). In order to carry out the study, the application of robust molecular methods was used.

MATERIALS AND METHODS

Type of study and location of sampling: A cross- sectional study was carried out between the March and July of 2015 in order to obtain the national frequency of *C. jejuni* and *C. coli* in poultry for human consumption. A sampling design was used in three levels of the Costa Rican poultry production chain: the farms, processing plants, and points of sale. This type of study was chosen because it allows estimation of the magnitude, and distribution of the pathogenic agent characterizing the sanity of poultry. All the poultry that was sampled during the investigation period (March – July 2015), from the three levels of the poultry chain, which was positive for *Campylobacter* spp by microbiologic culture was defined as case. The size of the sample required in order to determine national frequency was determined based on the frequencies of contamination reported by Zumbado *et al.* (2014). With the use of Win Episcope®, based on the formula used to determine frequency with an approximate population of 60 938 114 processed poultry in one year (Wright 2010) a frequency of 80% for lots containing fecal content, 40% carcass, and 50% at points of sale, assuming an acceptable error of 10% and a confidence level of 95%, it was determined that the required sample size was 63 poultry lot samples (126 of fecal content), 99 samples of carcass rinse, and 96 samples of points of sale, for a total of 321 samples. The number of samples acquired from the poultry lots was doubled (126) because two samples will be taken from each lot in order to avoid loss of material during analysis.

Farms: Broilers aged 35 through 40 days, weighing between 1500g and 3000g destined for human consumption were analyzed. The gross production of poultry in our country is concentrated in a few large scale producers who maintain strict biosecurity protocols, as well as management, and nutrition protocols for the fattening of broilers. Due to the fact that national poultry production is intense throughout the year, as well as *Campylobacter* spp characteristics, it was considered that the frequency f this pathogen doesn't vary according to production periods, or climate changes.

Retail stores: The sampling distribution for RS was done proportionally to the population size of each providence, using the three most populated cantons as reference, as well as the three most populated districts of each one. By doing this, it was assured that the samples would be obtained from those places where most of the product is commercialized, thus, those places where the largest amount of people could be exposed to *Campylobacter* spp.

Sample collection*:* In general, a total of 352 samples were collected and distributed according to the expected frequencies (Zumbado *et al.,* 2014) (n=152 farm samples, n=104 processing plant samples, and n=96 point of sale samples). Three types of samples were collected: cecum, in order to extract cecal content (CC) (sample which represents the farms), samples of carcass rinse after chiller (CA) (sample which represents the processing plants), and carcass rinse from retail stores (RS).

Cecal content: Poultry from each farm was identified at each processing plant. In this way, samples of CC and CA belonged to the same farm. The cecum from two fowls was collected immediately after evisceration. Each cecum was placed in a sterile plastic bag and identified accordingly. When a sample of cecal content was reported positive, it was inferred that the lot from which it came from was positive. This is due to the methodology in the production of poultry "All in, all out", and the characteristics of infection produced by *Campylobacter* spp. in these chickens. "All in, all out" means that all fowl from a same lot receive the same cares concerning vaccination, feeding, medications, amongst others. For this reason, it is highly probable that if this microorganism is present in one chicken, it's present in the rest of them as well.

Carcass rinse:Samples were taken from the carcasses through the rinse method (USDA, 2008) after the poultry had gone through the cooling process in the line of production. For each carcass sampled, internal temperature was taken from the depth of the breast with the use of a food- adequate thermometer which was properly calibrated and disinfected.

Carcass rinse acquired from points of sale: In RS, the sample that corresponds to carcass (known as clean chicken), was collected through the normal purchasing process. The sample was transported in the bag and conditions supplied by the retail store. As soon as the sample was received, internal temperature was taken from the depth of the breast with the use of a food-adequate thermometer which was properly calibrated and disinfected. Once the sample had reached the lab, each purchased carcass was rinsed as described previously. All of the collected samples were transported to the to the laboratory of Bacteriology of the School of Veterinary Medicine (EMV) of the National University (UNA), at a temperature no greater than 4°C in order for the respective analysis to take place.

Campylobacter spp. Culture: The rinsing process for the poultry carcass was taken from the ISO 10272-1:2006 protocol modified by the United States Department of Agriculture (USDA) (2008). Each sample was rinsed with 400 ml of buffered peptone water – BPW (Oxoid Ltd., Ogdensburg, NY). Afterwards, 30 ml of the rinse was taken and enriched in Preston enrichment broth base (Oxoid Ltd., Ogdensburg, NY). Following this process, incubation took place for 48 hours at 42±1 °C in microaerophilic conditions (10%O2, 5%CO2 and N2 for balance), using a Campygen generating system (Oxoid Ltd., Ogdensburg, NY). Consecutively, a 30 µl aliquot of the selective enrichment was transferred to the Modified Charcoal Cefoperazone Deoxychocolate- mCCDA agar plates (Oxoid Ltd., Ogdensburg, NY). After 48 hours of incubation, the presumptive colonies were confirmed at the genus level with the use of enzymatic tests (oxidase and catalase), and their microscopic morphology was determined. Those identified as *C. jejuni* and *C. coli* were kept at -80 °C (Farace and Viñas, 2007)-For the detection of *Campylobacter* spp. from the cecum samples, the cecal content was homogenized in 20ml of BPW.

1ml of the mixture was transferred to 9ml of Preston supplemented broth. The incubation period, time, and the biochemical screening methods were the same for the meat, and carcass of chicken.

Confirmation through species- specific PCR: The isolated samples which belonged to the *Campylobacter* spp. species were confirmed, and identified with multiple PCR using MapA, and CeuE genes with fragments of 589 and 462 bp for *C. jejuni*, and *C. coli* respectively (Denise *et al.,* 2001). The extraction of DNA was carried out using the boiling method (Denise *et al.,* 2001). Afterwards, 1 µl of DNA was added to PCR test tubes which contained 12.5 µl of GoTaq® Green Master Mix [1x], 20pmol of each primer, and nuclease- water for a final volume balance of 25 µl (Rahimi, 2011). The gene amplification was carried out following the protocol o thermal cycles used by Farace and Viñas (2007). The reference strains (*C. jejuni* ATCC 33560, and *C. coli* ATCC 33559) were employed as positive controls, *E. coli* ATCC25922 was used as negative control. Electrophoresis was carried out inagarose gel 1% stained with SYBR ®Safe (Invitrogen, USA). It was done at 75V, 400mA, and for 50 minutes, which was the time required for the differentiation of the marker band of 100pb (Ladder; Promega, USA). The gel was photo- documented on a Bio-Rad® transilluminator, and the bands were analyzed with the Chemi Doc System XRS software.

Statistical analysis: The frequency for *C. jejuni*, and *C. coli* was established both globally, and at each food chain level. Also, the frequency was calculated for each point of sale. Relative, and absolute frequencies were calculated, as well as qualitative nominal, ordinal, and discrete quantitative variables. Central tendency, and dispersion for the continuous quantitative variables were obtained. Info Stat® (Info Stat, FCA-UNC, Córdoba, Argentina), and Win Episcope 2.0 ® (EPIDECON, Zaragoza, Spain) were used for statistical analysis. The threshold value of significance (α) was established as 5%;

RESULTS

General aspects: A general frequency for *Campylobacter* spp. of 59.37% (209/352) was obtained.The frequency of *C. jejuni* was 42.85% (138/322),*C. coli* was 3.1% (10/322), and contamination of both species (mixed) was 8.69% (28/322).A total of 30 positive samples for the *Campylobacter* genus could not be identified as any species. Of the total strains whose species were identified through PCR, 78.40% (138/176) corresponded to *C. jejuni*, 5.68% (10/176) to *C. coli*, and 15.90% (28/176) to mixed contamination (Figure 1).

Cecal content: 152 samples of CC were collected. They represent the contamination status of the plant. These samples were obtained from a total of 76 farms, including 70 from the province of Alajuela, two from Heredia and Puntarenas, one from Cartago and San José. Farms analysis resulted in a 65,78% (n=50) frequency. *C. jejuni* was found in 28 of the sampled farms (44 positive samples). *C.* coli was found in four farms and mixed infection was found in 15 of them

Carcass Rinse: Out of all the carcass rinse samples analyzed (n=104), 64 (61.53%) of them were positive for *Campylobacter* spp. 53 (91.37%) of these corresponded to *C. jejuni*, three (5.17%) for *C. coli*, and two (3.44%) were mixed contamination.

Table 1. Geographic distribution of positive results at points of sale for *Campylobacter* spp, according to province, during March through July of 2015

Province (n)	Positives (% in province)	% Positives / RS total
Alajuela (19)	12 (63.15)	12.5
Cartago (12)	10 (83.33)	10.41
Guanacaste (7)	2 (28.57)	2.08
Heredia (10)	6 (60.00)	6.25
Limón (9)	5 (55.55)	5.20
Puntarenas (9)	1 (11.11)	1.04
San José (30)	22 (73.33)	22.91
Total (96)	58 (60.41)	60.41

Retail Sale: At this level of the food chain, frequency for *Campylobacter* was 60.42% (58/96); 41 samples (87.23%) were determined to be *C. jejuni*, one sample (2.12%) *C. coli*, and five samples (10.63%) mixed contamination. The frequency found at the central urban zone known as "Gran Área Metropolitana (Great metropolitan area) (GAM), was 78.57% (n=44/56); while a frequency of 37.50% (n=15/40) was found at the area outside of the GAM (rural area). Distribution by province is shown on Table 1. San José was the province with the highest isolation frequency followed by Cartago. Puntarenas was the province with the lowest isolation frequency.

DISCUSSION

Even though the number of samples obtained in the preliminary study done in Costa Rica in 2012 (Zumbado *et al.,* 2014) were limited, the nationwide results obtained in this study do not differ significantly. The frequency of *Campylobacter* spp. in samples obtained through carcass rinse after exiting the chiller heavily exceeds the results reported in previous studies, those being: 1.75% (Cid, 2010), and 0.00% (Rojas *et al.,* 1996). In both previous studies the microbiological isolation methods were different to the methods used in this study. In the case of the RS samples, the previous frequency reported was 63% (Antillon *et al.,* 1987) and 18% (Castrillo, 2010).

The samples were collected with a similar method from metropolitan areas. Results reported in Chile (54.00%) (Figueroa *et al.,* 2009), Jordan (61.5%) (Osaili *et al* 2012), and France (5.6%) (Denis *et al*., 2001) show similar frequency for CA. A similar situation occurs with RS, as the frequency is reported to be 58.3% in México (Zaidi *et al.,* 2012), 49.5% in Spain (Dominguez *et al.,* 2002), 76.0% in Maryland, USA (Cue *et al.,* 2005), 36.5% in Iran (Tareme *et al.,* 2006), and 87.5% in France (Hue *et al.,* 2010). In parallel, a frequency of 81.8% has been reported in samples obtained from commercial broiler farms in the south of Brazil (Kuana *et al* 2008); as well as 77.2% reported in the same type of samples obtained from France (Hue *et al.,* 2011) which is above the 57.24% obtained in this study. In any case, the frequency of *Campylobacter* spp. in the poultry for human consumption food chain is high. The highest frequency of positive results for *Campylobacter* spp. in CC, specially *C. jejuni*, was obtained from the province of Alajuela. Most of the broiler farms are located in those areas. Reciprocity was observed between the results obtained in CC, and CA in 21 (27.63%) of the cases. However, the frequency in CA exceeds the frequency in CC, even when strict hygiene

controls, such as the use of chlorine, and/or peracetic acid in the cooling water, reduction in temperature, implementation of GHMP, or HACCP are used at the processing plants. It must be taken into account that during the cooling process, carcasses are maintained together in the cooling water. For this reason, contamination could disseminate through this process. It has been shown that carcasses that belong to poultry lots which are CC positive for *Campylobacter* presented a higher UFC per gram than those which are CC negative (Rosenquist *et al.,* 2006*,* Allen *et al.,* 2007, Reich *et al.,* 2008, Hue *et al.,* 2011). This shows that evisceration in the processing plants is the step in the food chain that contributes the most to disseminating contamination (Hue *et al* 2010, Hue *et al.,* 2011). For this reason, reduction in the intestinal colonization of broiler could considerably reduce the contamination of the final product, and thus, the possibility of human campylobacteriosis (Fischer *et al.,* 2013). It has been described that those lots that do not sacrifice first thing in the morning schedule have a higher risk of being positive for *Campylobacter*. Other risk factors associated to positive results for Campylobacter that should be taken into consideration are maintaining evisceration room temperature higher than 15°C, and visible contamination of the evisceration (Hue *et al.* 2010).

In this investigation, a mild rise in the frequency of CA in relation to RS was demonstrated, contrary to what was expected according to the results in the preliminary study (Zumbado *et al.,* 2014).However, a similar behavior was reported in France (Denis *et al.,* 2001). None the less, the frequency in RS is high, and this could be due to a flaw in the transportation of poultry, and maintenance of adequate conditions at the RS, such as higher temperature, dirty cold display chambers, presence of flies close to the product, contact of the boxes containing the products with the ground, amongst other causes observed during visits. A higher frequency of positive results was observed at RS within the GAM with respect to rural areas. This reflects that an irregular manipulation could exist once received in the metropolitan area. For example, crossed contamination with other animal products could be a cause due to the fact that no significant association was made with the origin of the product. In a near future, the goal for food safety authorities should be the reduction in *Campylobacter* spp. in the food chain (Macé *et al., 2016*). This objective could be reached by maintaining active surveillance for this infectious agent in the distinct areas of the food chain, as well as evaluating the efficacy of these chemical, or physical interventions. The frequency of *Campylobacter* spp found in this investigation is high, and it represents a danger for the public health of Costa Rica. Preventative measures against this infectious agent are scarce, and deficient, which results in high levels of contamination of food products.

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REFERENCES

Allen V M., Bull SA, Corry JEL, Domingue G., Jorgensen F., Frost JA., Whyte R., Gonzalez A., Elviss N. and Humphrey TJ. 2007. *Campylobacter* spp. contamination of chicken carcasses during processing in relation to flock colonisation. *Int.J. Food Microb*., 113: 54-61.

Antillón F., Odio E. and García V. 1987. Presencia de *Campylobacter jejuni, C. coli* y *C. laridis* en pollos frescos del área Metropolitana de San José, Costa Rica. Rev. Cost. Cienc. Méd. 8: 39-41.

Castrillo N. 2010. Comparación de 3 técnicas para la detección de *Campylobacte*r en muestras de pollos frescos comercializados en el área Metropolitana. (Tesis de Licenciatura). Universidad de Costa Rica. San José.

Cid A. 2010. Determinación de la presencia de *Campylobacter* spp en pollo y sus derivados, en la provincia de Alajuela. (Tesis de Licenciatura). Universidad de Costa Rica. San José.

Centers for Disease Control and Prevention. 2010. *Campylobacter*: General information. Disponible en http://www.cdc.gov/nczved/divisions/dfbmd/diseases/campylobacter. Revisado 19 de enero 2016.

Cui S., Ge B., ZhengJ and Meng J. 2005. Frequency and Antimicrobial Resistance of *Campylobacter s*pp. and *Salmonella* Serovars in Organic Chickens from Maryland Retail Stores. *Appl. Envirom Microb*. 71: 4108–4111.

de Boer P., Rahaoui H., Leer RJ., Montijn RC. and van der Vossen JM. 2015. Real-time PCR detection of *Campylobacter* spp.: A comparison to classic culturing and enrichment. *Food Microb*. 51: 96–100.

Denis M., Refregier-Petton J., Laisney MJ., Ermel G. and Salvat G. 2001. *Campylobacter* contamination in French chicken production from farm to consumers. Use of a PCR assay for detection and identification of *Campylobacter jejuni* and *Camp. coli*. J. Appl. Microbiol. 91: 255–267. http://doi.org/ 10.1046/j.1365-2672.2001.01380.x

Domínguez C., Gómez I. and Zumalacárregui J. 2002. Frequency of *Salmonella* and *Campylobacter* in retail chicken meat in Spain. *J. Food Microb*. 72: 165-168.

Ebrahim Rahimi M.A. 2011. Antimicrobial resistance patterns of *Campylobacter* spp. isolated from chiken, turkey quail, partridge, and ostrich meat in Iran. Food Control. 22: 6.

European Food Safety Authority y European Centre for Disease Prevention and Control. 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. EFSA Journal 13: 4329. doi:10.2903/j.efsa.2015.4329

Farace MI. and Viñas MR. 2007. Manual de procedimientos para el aislamiento y

caracterización de Campylobacter spp. WHO Global Salm Surv/OPS. Disponible en: http://bvs.panalimentos.org/local/file/Manual_*Campylobacter*_31-08-2007.pdf. Revisado 23 enero 2016.

Figueroa G., Troncoso M., López C., Rivas P. Toro M. 2009. Occurrence and enumeration of *Campylobacter* spp. during the processing of Chilean broilers. *BMC Microbiology*, 9: 94.

Fischer S., Kittler S., Klein G. and Glünder G. 2013. Microplate-test for the rapid determination of bacteriophage-susceptibility of *Campylobacter* isolates development and validation. PLoS ONE, 8: 52

Hue, O., Le Bouquin S., Laisney MJ., Allain V., Lalande F., Petetin I., Rouxel S., Quesne S., Gloaquen PY., Picherot M., Santolini J., Salvat G., Bougeard S. and Chemaly M. 2010. Frequency of and risk factors for *Campylobacter* spp. Contamination of broiler chicken carcasses at the slaughterhouse. *Food Microbiol.* 27: 992-9.

Hue O., Allain V., Laisney MJ., Le Bouquin S., Lalande F., Petetin I., Rouxel S., Quesne S., Gloaquen PY., Picherot M., Santolini J., Bougeard S., Salvat G. and Chemaly M. 2011. *Campylobacter* contamination of broiler caeca and carcasses at the slaughterhouse and correlation with Salmonella contamination. *Food Microbiol*. 28: 862-8.

Kuana S., Santos L., Rodrigues L., Borsoi A., Moraes H., Salle C. and Nascimento V. 2008. Ocurrence and characterization of *Campylobacter* in the Brazilian production and processing of broilers. *Avian Diseases* 52: 680-684.

Leonard E., Tompkins L., Falkow S. and Nachamkin I. 2004. Comparison of *Campylobacter jejuni* isolates implicated in Guillain-Barré Syndrome and strains that cause enteritis by a DNA microarray. Infect Immun. 72: 1199–1203.

Macé S., Haddad N., Zagorec M. and Tresse O. 2016. Influence of measurement and control of microaerobic gaseous atmospheres in methods for *Campylobacter* growth studies. *Food Microbiol*.52: 169–176.

Osaili T., Alaboudi A. and Al-Akhras R. 2012. Frequency and antimicrobial susceptibility of *Campylobacter* spp. in live and dressed chicken in Jordan. *Foodborne Pathogens and Disease,* 9: 54-58.

Pacheco K. and Peña Y. 1995. Determinación de *Campylobacter* sp en pollos de consumo humano. (Tesis de Licenciatura). Universidad de Costa Rica. San José.

Reich F., Atanassova V., Haunhorst E. and Klein G. 2008. The effects of *Campylobacter* numbers in caeca en the contamination of broiler carcasses with *Campylobacter*. *Int. J. Food Microbiol.* 127: 116-120.

Rojas X., Rojas Y., Soto L., Delgado D and . Hernández F. 1996. *Campylobacter* sp. en pollos para consumo humano. *Rev. Cost Cienc. Med.* 17: 34.

Rosenquist H., Sommer HN., Nielsen NL. and Christensen BB. 2006. The effect of slaughter operations on the contamination of chicken carcasees with thermotolerant *Campylobacter. Int. J.Food Microbiol*., 108: 226-232.

Taremi M., Mehdi M., Gachkar L., MoezArdalan S., Zolfagharian K. and Reza M. 2006. Frequency and antimicrobial resistance of *31.* isolated from retail raw chickens and beef meat, Tehran, *Iran. Int. J. Food Microbiol.* 108: 401403.

United States Departament of Agriculture. 2008. MGL 4.04:2008. Isolation and identification of *Salmonella* from meat, poultry and egg products. Georgia, USA.

Wright C. 2010. La industria avícola costarricense. Consultado: 21 de febrero del 2013 http/www.wattagnet.com

Zaidi M., McDermott P., Campos F., Chim R., León M. and Vázquez G. 2012. Antimicrobial-Resistant *Campylobacter* in the Food Chain in Mexico. *Foodborne Pathogens and Disease*. 9: 841-847.

Zumbado-Gutiérrez L., Arévalo-Madrigal A., Donado-Godoy M., and Romero-Zúñiga J. 2014. Diagnóstico molecular de *Campylobacter* en la cadena avícola destinada para consumo humano en Costa Rica. *Agronomía Mesoamericana*. 25: 357.

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