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Presencia de patógenos en carne cruda de pollo en centros de expendio, Huánuco-Perú: una problemática en salud

Presence of pathogens in raw chicken meat in retail centers, Huánuco-Peru: A health problem

Presença de patógenos na carne crua de frango em centros comerciais, Huánuco-Peru: um problema de saúde

Vásquez-Ampuero Juan Marco *, Tasayco-Alcántara Walter Richard

Article Info

Hermilio Valdizan National University.
Faculty of veterinary medicine and zootechnics.
Professional School of Veterinary Medicine.
Pillco Marca, Av. Universitaria 601-607.
Cayhuayna – Huanuco.
Perú.
Tel: (062) 59 1060-591082
wtasaycoalcantara@gmail.com

*** Contact address**:
Hermilio Valdizan National University.
Faculty of veterinary medicine and zootechnics.
Professional School of Veterinary Medicine.
Pillco Marca, Av. Universitaria 601-607.
Cayhuayna – Huanuco.
Perú.
Tel: (062) 59 1060-591082
Móvil: 000000000.

Juan Marco Vásquez-Ampuero
E-mail address: jmarcovasqueza@gmail.com

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Resumen

El objetivo de este estudio fue determinar la condición higiénica sanitaria de los centros de expendio de carne de pollo (CP) crudo de los mercados principales de la ciudad de Huánuco-Perú. Se muestrearon 50 establecimientos que expenden CP cruda. En cada uno de los locales se tomaron muestras de 200 g de carne, las cuales fueron procesadas en el LM-FMVZ UNHV. Para la detección de *Escherichia coli*, se empleó placas Petrifilm EC de acuerdo al método oficial y para *Salmonella* spp., el ensayo Salmonella Express System (SALX) en placas 3M Petrifilm. Paralelamente, para evaluar las condiciones higiénicas sanitarias de los establecimientos y de los responsables del expendio de carne, se aplicó un cuestionario de buenas prácticas de manipulación. Se observó que todas las muestras se encontraban contaminadas, con UFC/g PF de *E. coli*, en promedio 1988 UFC/g PF±3.74 de *E. coli*. Y en cuanto a *Salmonella* spp., todos los casos fueron positivos a *la misma.* Además, se demuestran condiciones higiénicas sanitarias deficientes y regulares de infraestructura. Por tanto, el expendio de CP cruda no cumple con las buenas prácticas de manipulación de alimentos. En los mercados de Huánuco, la CP expendida está considerada por la NTP no es apta para el consumo humano.

Resumo

O objetivo deste estudo foi determinar as condições higiênicas sanitárias dos centros de vendas de carne crua de frango (CF) nos principais mercados da cidade de Huánuco-Peru. Foram amostrados 50 estabelecimentos que vendem CF bruto. Foram coletadas 200 g de amostras de carne em cada uma das instalações, processadas no LM-FMVZ UNHV. Para a detecção de *Escherichia coli*, as placas Petrifilm EC foram usadas de acordo com o método oficial e para *Salmonella* spp., O ensaio Salmonella Express System (SALX) em placas 3M Petrifilm. Ao mesmo tempo, para avaliar as condições sanitárias e higiênicas dos estabelecimentos e dos responsáveis pela venda de carne, foi aplicado um questionário sobre boas práticas de manuseio. Observou-se que todas as amostras estavam contaminadas, com UFC / g PF de E. coli, em média 1988 UFC / g PF±3.74 de *E. coli*. E quanto a *Salmonella* spp., Todos os casos foram positivos. Além disso, são demonstradas más e regulares condições de higiene sanitária da infraestrutura. Portanto, a venda de matéria-prima bruta não cumpre as boas práticas de manuseio de alimentos. Nos mercados de Huánuco, o CF emitido é considerado pelo NTP inadequado para consumo humano.

Keywords:

Enterobacteriaceae,
Salmonella,
Escherichia coli,
chicken meat,
hygiene practices,
markets,
sanitary conditions.

Abstract

The objective of this study was to determine the sanitary hygienic condition of the raw chicken meat (CM) sales centers in the main markets of the city of Huánuco-Peru. 50 establishments that sell raw CM were sampled. Samples of 200 g of meat were taken in each of the premises, which were processed in the LM-FMVZ UNHV. For the detection of *Escherichia coli*, Petrifilm EC plates were used according to the official method and for *Salmonella* spp., The Salmonella Express System (SALX) assay on 3M Petrifilm plates. At the same time, to assess the sanitary and hygienic conditions of the establishments and those responsible for selling meat, a questionnaire on good handling practices was applied. It was observed that all the samples were contaminated, with CFU / g PF of *E. coli*, on average 1988 CFU / g PF±3.74 of *E. coli*, and as for *Salmonella* spp., all the cases were positive to it. In addition, poor and regular sanitary hygiene conditions of infrastructure are demonstrated. Therefore, the sale of raw CM does not comply with good food handling practices. In the Huánuco markets, the issued CM is considered by the NTP to be unfit for human consumption.

Introduction

Foodborne diseases (FBD) are caused by swallowing food or water contaminated with microbiological agents in such quantities that they affect the health of the consumer at the individual level or in groups of people and that the infection may correspond to a lack of production, handling, maintenance, shipping, distribution, marketing and sale of food and water[1]. In fact, it is difficult to estimate with conviction the occurrence of FBD worldwide and nationally, the relevance of the problem is unquestionable due to the number of people affected or who die from eating food not suitable for consumption. However, the objective extent of the problem continues to be ignored since the generality of the cases presented is not reported.

On the other hand, contamination of raw meat products is very common, due to the presence of enteric bacteria that naturally inhabit the digestive tract of many organisms for human consumption. These agents are potentially pathogenic and their detection is relevant according to official regulations for this type of product.

Currently one of the most consumed foods, is the chicken meat (CM), for its low cost compared to other meat products, its appetizing taste, the versatility in its different preparations and healthier than other meat products[1,2]. However, this food can be a carrier of infections such as salmonellosis, listeriosis, campylobacteriosis, as well as enterobacteria such as *Escherichia coli (E. coli)* ST131 and *Yersinia pseudotuberculosis*, known as FBD[3,4]. Hence, the importance of carrying out good production practices (GPP), processing, transportation, and retail centers for sale. This chain of events must guarantee safety in order to provide a product in optimal conditions[2,5].

Food handling (FH) plays relevant roles in the incidence and prevalence of FBD, being a cause of gastrointestinal disorders, which can determine symptoms such as acute diarrhoea[4,6]. Therefore, for the state, at the level of public health, they are im-

portant since they are determinants of morbidity[3]. The World Health Organization (WHO) estimates that approximately 600 million people worldwide become ill from eating contaminated food, of which 420000 die from this cause, with about 125000 children under the age of five[3].

In this sense, some of the measures for the correct hygienic handling of food involve hand washing, refrigeration, and cooking of meat, which are essential to prevent the transmission of these diseases[4,6]. Hence, raw meat must be handled with care to avoid contamination, such as cross-contamination, especially when pathogens are transmitted through the juices of raw birds when they come into contact with other foods[5], and of course due to poor hygiene practices during human handling[7].

Fresh chicken (FC) is distributed complete, or in most cases, by parts, and when offered separately, the profits for the distributor are much higher, in a way, customers get the piece they want. For many, the FC represents an option, since frozen, is a type of distribution that requires a cold chain, very specific to ensure their freshness, therefore, the supply centers must have the equipment to ensure these processes. Retail distributors of frozen chicken (FZC), acquire from large companies, the mechanics of buying FC and FZC is not allowed, since it may take a long time to sell an FZC and the possibility of contamination increases due to the number of potential microorganisms for FBD[8].

The types of microorganisms that can cause disease can be viruses, bacteria, fungi, and parasites. Bacteria are responsible for more than 90% of confirmed cases of FBD, highlighting 5 bacteria associated with FBD, the most frequent are: *Salmonella* (not typhoid), *E. coli* ST131, *Listeria monocytogenes*, and *Campylobacter* spp[3,9].

Bacterial populations that may be present in the CM come from the animal's gastrointestinal tract and those that are added during the handling of the bird from its slaughter to its sale to the public[4,9]. In Peru, the commercialization of chicken is very varied, but in general, due to the idiosyncrasy of the population, the meat is not handled under good hygiene practices (GHP)[10].

The reason why this study aims to determine the sanitary hygienic condition of the raw chicken meat sales centers of the main markets of the city of Huánuco-Peru.

Materials and methods

A descriptive cross-sectional study was carried out, in which five poultry markets in the city of Huánuco, Peru were chosen at random, in each one, 10 establishments selling FC were randomly considered. In each one, a bulk sampling was performed, taking an approximate weight of 200 g of crude CM, following the instructions of the Peruvian Technical Standard (PTS) 201.054: 2001 of the Ministry of Health[11] (table 1). Sampling was done on a weekly basis starting in June and culminating in November 2019. In addition, a semi-structured observation guide was applied to each of the premises to evaluate the marketing conditions of each sampling location.

Bacteriological evaluation. In accordance with PTS 201.054: 2001, non-aseptic samples were randomly taken, directly from the retail sites, from the same batch presented to the consumer, and which were exposed to the open air[11,12]. These were placed in

hermetically sealed plastic bags and then stored in a cooler with drinking ice, ensuring a temperature between 2 and 8 °C, and then immediately transported to the Microbiology Laboratory of the Faculty of Veterinary Medicine and Zootechnics (ML-FVMZ) of the National University Hermilio Valdizan (NUHV), for their respective analysis.

Table 1 Number of establishments for the sale of raw chicken meat belonging to the poultry markets of the city of Huánuco evaluated in the study

Markets of the city of Huánuco	Number of establishments evaluated	Market coding
Market Modelo	10	1
Market Paucarbamba	10	2
Market Antiguo	10	3
Market de las Moras	9	4
Market Pillcomarca	11	5
Total establishments	50	

The samples analyzed are homogenates of 25 g and were made in five times. In the case of *E. coli*, the number of colonies obtained was multiplied by the corresponding dilution factor, the results being expressed in colony-forming units per gram of fresh chicken (CFU/g FC), while in the case of *Salmonella* spp., only presence or absence is expressed per 25 g sample as established by the PTS[11,12].

To determine the presence of *Escherichia coli (E. coli)* and total coliforms, under a laminar flow hood, EC Petrifilm plates were inoculated with 1 mL of the samples according to the official method and for their reading, the colonies that presented a blue or bluish red color associated with gas bubble formation by lactose fermentation were counted for *E. coli* and red colonies for the cases of the other coliforms[13]. It is important to note that, all materials were placed inside the laminar flow booth to avoid external contamination.

The presence of *Salmonella* spp. was determined using the Salmonella Express System (SALX) test on 3M Petrifilm plates[13], which allows a rapid biochemical and qualitative detection that confirms the presence of bacteria of the *Salmonella* genus. This presents a chromogenic culture medium ready to use that contains a gelling agent soluble in cold water, selective and differential for *Salmonella*, providing a presumptive result, which later is corroborated with the use of the SALX disc that presents a substrate that facilitates the biochemical confirmation of the genus.

Health Assessment. In the poultry retail establishments selected for sampling, a record was made of the identification data of the market stall, the owner and/or food handler, as well as the hygienic-sanitary characteristics of the premises and the product handlers. A structured questionnaire was used for this purpose, which summarized socio-demographic data on the owner and/or vendor, the hygienic-sanitary characteristics of the handler and place of sale, as well as the characteristics of the CM sale, in terms of maintenance and conservation of the meat.

The data were processed using descriptive statistics by frequency distribution.

Results

Table 2 presents the results regarding the presence of *E. coli* and *Salmonella* spp in the raw chicken meat sold in the market establishments in the city of Huánuco, which shows that in 28 % of the establishments analyzed the result was positive for *E. coli*, while in 72% the result was negative in the samples of the 50 establishments studied. It is relevant to highlight that, in all positive cases, CFU/g FC of *E. coli* was observed, on average 1988 CFU/g FC±3.74 of *E. coli* (figure 1).

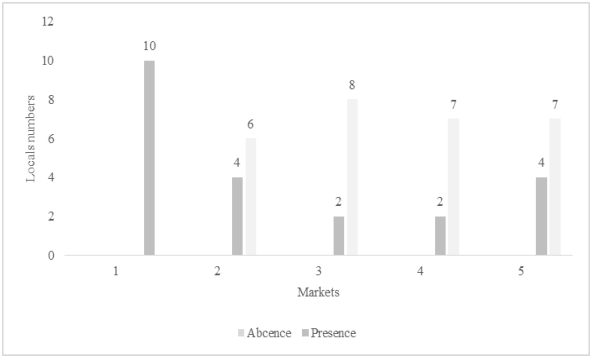
On the other hand, regarding *Salmonella* spp, the PTS indicates as an acceptable limit, absence/25 g (figure 2), and reason why, in 62% of the evaluated establishments were positive to *Salmonella* spp, with an average of 35.88 CFU/g and only 38% resulted negative to it.

Table 2 Presence of *E. coli* and *Salmonella* spp in raw chicken meat that is sold in establishments in the markets of the city of Huánuco

	E. coli		***Salmonella* spp**	
	Frequency	Porcentage	Frequency	Porcentage
Positive	14	28	31	62
Negative	36	72	19	38
Total	**50**	**100**	**50**	**100**

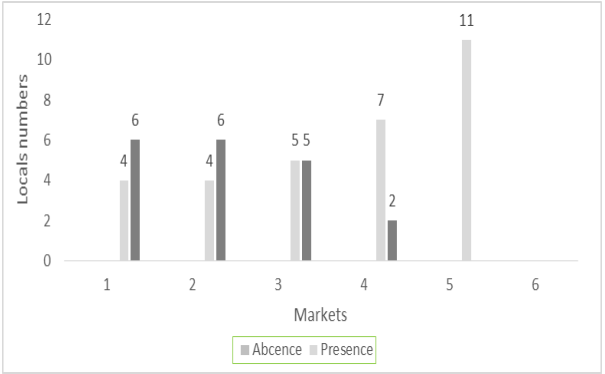
Regards to the evaluation of the application of good handling practices (GHP) by the owners, vendors or handlers of meat by each establishment, it was found that 34 (68%) declared to have studies at the secondary level, 13 (26%) completed primary education and only 3 (6%) had studied at a higher level. Thus, 86% (n= 43) were female, and 16% (n= 8) indicated that they had received training and/or instruction in handling raw chicken meat.

Figure 1 Presence of *Escherichia coli* in raw chicken meat that is sold in market establishments in the city of Huánuco



When evaluating the general conditions of maintenance of the infrastructure, as well as the hygiene conditions of the establishments (figure 3), it was observed that more than 50% were observed in regular conditions. The 50% (n=25) of the establishments stated that they do not have potable water for cleaning surfaces and personnel.

Figure 2 Presence of *Salmonella* spp in raw chicken meat that is sold in the establishments of the markets of the city of Huánuco



Regarding the handling of chickens, figure 4 shows that 90% (n=45) of the personnel do not use gloves

and 96% (n=48) are paid directly with their hands, among which 28% (n=14) had dirty hands and 58% (n=29) long nails and about protective gear, even though the majority 78% (n=39) wear aprons, only 4% (n=2) use caps.

Figure 3 Hygienic sanitary conditions and states of the infrastructure of establishments for the sale of raw chicken meat in the markets of the city of Huánuco

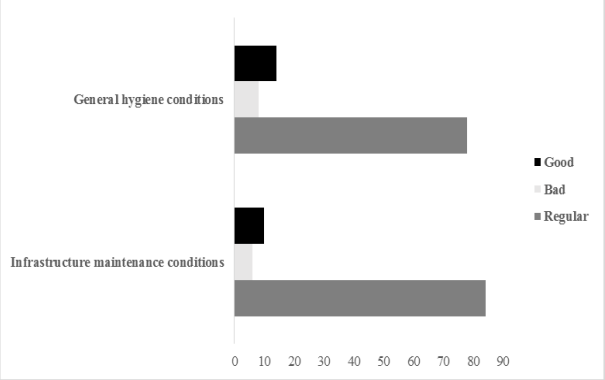
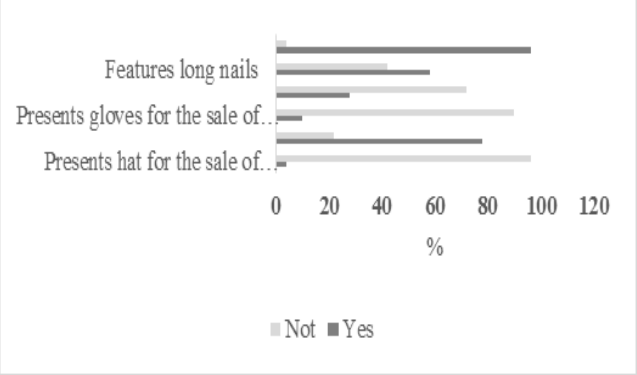
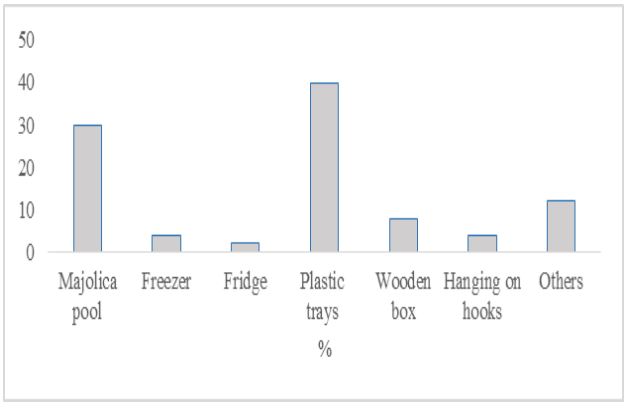


Figure 4 Management of good sanitary practices of the raw chicken meat handler, in each of the establishments belonging to the markets of the city of Huánuco



Likewise, Figure 5 shows that most of the establishments evaluated, keep the meat on the majolica pools, and in plastic trays in 30% and 40% respectively, only 1 (2%) establishment had a refrigerator and two (4%) with freezers.

Figure 5 Hygienic and sanitary conditions for storing raw chicken meat in each of the establishments belonging to the markets of the city of Huánuco



Discussions

The hygienic-sanitary conditions of 50 establishments that sell raw CM distributed in five poultry markets in the city of Huánuco - Peru were evaluated, of which 28% (n=14) of them, the raw chicken meat was positive to *E. coli*, with counts above what is established by the PTS 201.054, which determines 500 CFU/g as the upper limit. Likewise, *Salmonella* spp. was observed in most establishments, an average of 35.88 CFU/g, and considering the PTS as a limit, absence for each 25 g of sample, in this study, 62% of the establishments evaluated are above what is available as an acceptable level for consumption[11].

The presence of these bacterial contaminants is a warning call, since the high risk of intestinal poisoning that causes the acute diarrheal disease is known[13]. Consumers of CM who attend these markets to acquire the product are exposed to risk. These results are compared with two similar studies, one also carried out in the markets of Huánuco, where the presence of *E. coli* was determined in 64% of the evaluated premises and another in the

markets of Jaén, in the Department of Cajamarca, Peru, which reported the presence of both *E. coli* and *Salmonella* in raw chicken meat sold in points of sale, with similar infrastructure to those of the city of Huánuco, with levels as high as 57 x10^7 CFU/g of *E. coli* and the presence of *Salmonella* in 57% of the premises evaluated[10,14,15], likewise Lucas et al.[6] in the city of Lima, reported the presence of pathogenic strains of *E. coli* in 68% of the premises.

Studies carried out in other countries, which are described below, also report the presence of these cases presented by López et al.[16], raises 14% of *E. coli* and 56% of *Salmonella* in the raw and ground meat sold in the 47 supermarkets evaluated in the municipality of San Salvador and Mejicano, in El Salvador. Araujo- Guerra[5], evaluated stalls in outdoor markets in Valledupar, Colombia, reported a prevalence of 18% of *Salmonella*. Likewise, similar results are reported in studies carried out in India, Egypt, and Sri Lanka, when evaluating samples of raw chicken meat sold in outdoor market stalls, finding prevalence of 14 to 45% for *E. coli* and 12 to 56% prevalence for *Salmonella*[7,17-20]. In all cases, the common factor is the style of sale, establishments that do not allow the continuity of the refrigeration chain, and minimum handling of the product.

The contamination observed in raw chicken meat may be due to cross-contamination, it is enough that one specimen is contaminated, due to the type of handling that this product receives, in this form of street sale, where the conditions of refrigeration and cleaning of the tables and work utensils are not appropriate, the temperature and general form of handling are determining factors for the proliferation of these bacteria[5,6,21,22]. Villalpando-Guzmán et al.[23] observed that the presence of *Salmonella* spp. in samples of chicken meat increases when it is processed, whether it is sold in pieces or ground.

Studies such as those by Alvarado-Lagunas et al.[2], Lucas et al.[6], Zakki et al.[24] and Molina et al.[25], point out that the hygiene of the facilities where the slaughter is carried out, the outdoor markets and the personal hygiene of the workers in the processing plants, until it reaches the retailer, due to poor hygienic conditions, promotes the growth of contaminants that make chicken meat unsafe for consumption. In this sense, in this study, an evaluation was carried out on the minimum compliance with standards according to GHP, where it was observed that, out of 50 establishments, 16% (n=8) indicated that they had received training and/or instruction in handling raw chicken meat. On the other hand, when evaluating the general conditions of infrastructure maintenance, as well as the hygiene conditions of the establishments, more than 50% of them were in regular conditions. Declaring 50% (n=25) of the establishments, which do not have potable water for the cleaning of surfaces and personnel. In addition, in the evaluation of good practices, 90% (n=45) do not use gloves and 28% (n=14) have dirty hands and 58% (n=29) have long nails. It is evident that the hands are the main route of product contamination. Only in three cases was it observed that the person receiving payment is different from the person handling the product. Likewise, out of 50 establishments, only one had a refrigerator and two had freezers, so these chicken shops do not have the necessary conditions to keep the chicken meat at an adequate temperature during working hours. This is an important measure since, in the case of Salmonella, it has been demonstrated that wooden boards and storage containers[26], it has been demonstrated that wooden boards and storage containers, and

even the water used in rinsing can act as the main routes of cross transmission[22,23].

The bacterial counts reported in this study are to be expected given the observed sanitary-hygienic conditions[10]. It is evident that the contamination of the samples is crossed since the personnel, even though most of them have completed their secondary education, not all have received GHP training and it is increased due to inadequate maintenance of the product since the cold chain is not complied with and the chicken meat is exposed to the environment for a long time[13,27,28].

Escobedo-Bailón & Martel-Tolentino [10], in his study in the markets of the city of Huánuco, also concluded that the hygienic-sanitary conditions of these are deficient, reporting bacterial contamination, pointing out that these establishments do not comply with the GHP required by the current PTS. The hygienic-sanitary conditions of both facilities and personnel significantly affect the counts of contaminating bacteria, including *E. coli* and *Salmonella*[24].

Khan et al.[29] carried out a study in Trinidad and Tobago, comparing the handling of refrigerated raw chicken meat, such as that of supermarkets, and that kept at room temperature during working hours, from artisan markets that process poultry directly from the corral, and observed a higher prevalence of *Salmonella* in those sales premises that did not keep the product refrigerated. The cooling conditions for *Salmonella*, are determinant as it has been reported to proliferate even at temperatures around 4 °C[5,13], Kulasooriya et al.[19] evaluating raw and cooked chicken meats in refrigeration, found prevalence's of 21% and 10% for *Salmonella* and *E. coli* respectively, in raw condition compared to 8% when they were cooked. Likewise, Ibrahim et al.[30] reported contamination with *E. coli* and *Staphylococcus aureus* in most raw and frozen prepared chicken meat products. Hence, the importance of maintaining this type of product in a low-temperature environment to ensure its safety for consumption.

In general terms, the fact that *E. coli* and *Salmonella* are part of the intestinal flora of many organisms, including birds, means that these bacteria represent a high risk since they can potentially be highly pathogenic, threatening the health of consumers and because they can contaminate meat and other foods if basic hygiene conditions are not maintained.

The finding of these two species in the samples analyzed, becomes worrisome for the public health of the population of the city of Huánuco therefore, environmental social responsibility is required to monitor not only compliance with the standard but also to carry out bacteriological evaluations to ensure safety. To carry out this, it is necessary to monitor the hygiene conditions of the facilities where the birds are slaughtered up to those of the outdoor market[26], and in turn, ensure compliance with the personal hygiene of the workers in each of the processes through which the product passes, in order to ensure that the products are fit for consumption[20,24,31].

Taking into account all these studies, the importance of proper cooking of the CM and of all meat products to be consumed with the minimum risk[19,21], in addition to adequate hygiene practices[31,32]. However, in the case of Peru, it is part of the idiosyncrasy to believe that the chicken that is sold in this type of premises (without refrigeration and without evisceration) is fresh, which guarantees them a low risk of contaminants, as well as offering them a better quality of meat in terms of softness and flavor, so that even when the consumer observes the conditions of

these establishments, his confidence in the quality of the product persists[6].

On the other hand, based on the Escobedo-Bailón & Martel-Tolentino[10] study, a relation is inferred between the way in which chicken meat is sold and contamination by micro-organisms, with a potential risk to public health. Hence, the government entities responsible for ensuring that meat shops comply with the minimum health conditions and that they offer products that are suitable for consumption, not only direct their efforts towards ensuring that these standards are met, but also implement training strategies in schools and through advertising campaigns, informing consumers of the risks associated with the improper handling of meat products, thus educating the consumer, who ultimately will also play the role of overseeing compliance with the standard.

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Conflicts of interest

The authors declare that there is no conflict of interest in the development of this research work.

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Ethical aspects

This study was approved by the Ethics Committee of the Vice-Rectorate of Research of the National Hermilio Valdizan University (Huánuco, Peru), in accordance with the ethical standards in line with the Declaration of Helsinki 1975, modification 1983. Thus, the establishments belonging to the markets and poultry farms of the city of Huánuco, submitted to the sanitary hygienic evaluation in the present study, were processed under a codification for the protection of the participating personnel as owners and/or food handlers, who voluntarily agreed to participate in the study, signing an informed consent.

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