Food Protection Trends, Vol. 25, No. 7, Pages 522–530

6200 Aurora Ave., Suite 200W, Des Moines, IA 50322-2864

International Association for Food Protection

Use of a GMP/GHP HACCP Checklist to Evaluate the Hygienic Status of Traditional Dry Sausage Workshops

SILVINA FADDA,[1] TERESA AYMERICH,[1] MARTA HUGAS,[2] and MARGARITA GARRIGA[1]*
[1]CERELA – CONICET, Chacabuco 145, 4000 San Miguel de Tucumán, Argentina; [2]European Food Safety Authority (EFSA), Largo N. Palli 5/A, I-43100 Parma

SUMMARY

The purpose of this work was to evaluate the hygienic status and the feasibility of implementing a self-control system in ten traditional dry sausage workshops in Catalonia (Spain). A Good Manufacturing and Hygienic Practice checklist based upon HACCP principles was incorporated into a questionnaire. It included topics related to pre-requisites of a self-control system, critical points of the process and the efficiency of the hygiene program used at their facilities. In addition, analyses of spoilage/pathogen flora in environmental samples and products, as well as measurements of temperature and relative humidity, were carried out at several facilities. After the questionnaire had been completed, traditional workshops were ranked. All workshops studied presented adequate infrastructures for implementation of a self-control system. In general, cold rooms and mixing machines were classified as "ultraclean" (0–2 × 10^2 *Enterobacteriaceae* CFU/100 cm^2) and no pathogens were detected in them. Stuffing machines received a "not clean" denomination (> 10^3 *Enterobacteriaceae* CFU/100 cm^2), with *Listeria monocytogenes* present in 20% of these. Pathogen concentrations of dry sausages from all workshops studied were below pre-established limits (*Salmonella* and *Escherichia coli* verotoxigenic (VTEC): not detected in 25 g; *L. monocytogenes*: < 100 CFU/g; *Staphylococcus aureus*: < 500 CFU/g). All producers reached a "sufficient" classification according to the criteria established, although some aspects (high temperature, low humidity of meat reception/storage areas, excessive time for casing desalting, and presence of *L. monocytogenes* in some machines) should be corrected. A systematic application of this kind of HACCP checklist could help small producers to improve the hygienic quality of their facilities and products.

A peer-reviewed article

*Author for correspondence: +34972630052; Fax: +34972630373
E-mail: margarita.garriga@irta.es

INTRODUCTION

Food quality, rather than quantity, is now the priority in Europe. High quality could be assured by sustainable agricultural production, which should also take into account the concerns of consumers, particularly with regard to food quality, food safety and traditional/organic production methods.

In the meat sector, the recent BSE crisis and recurring food poisoning cases have undermined public confidence in intensive and industrial meat production. Consumers are therefore turning to "traditional" products, and the growth in sales of natural and organic foods is clear evidence of this. Traditional fermented dry sausages account for a significant part of this domain.

Production of traditional dry sausages relies on natural "contamination" by environmental flora. This contamination occurs during slaughter and increases during manufacture. Each workshop has a specific house flora, composed of useful microorganisms responsible for the fermentation and flavor of sausages, as well as spoilage and pathogenic flora. The few studies that have been conducted on traditional meat products have shown that hygienic shortcomings can lead to a production loss of up to 25%, with serious economic consequences *(22, 28)*. It is crucial, therefore, to enable traditional producers to manufacture products that are safe and standardized while retaining their typical sensory qualities.

The implementation of the Hazard Analysis and Critical Control Points (HACCP) concept in food production facilities is internationally recognized as an effective way to ensure the safety of food products *(32)*. HACCP systems involve a proactive rather than a retroactive approach. For maximum preventive effect, Quality Assurance (QA) programs need to cover the whole production chain, from "farm to fork", thereby including all aspects of the process, from the rearing of the animals to food preparation practices of the final consumer. QA programs aim to control, prevent or eliminate problems, and ideally should start with the eradication of microbial pathogens from farm animals. Continuous monitoring of the whole production process ensures that control measures can be introduced promptly and effectively in response to either new hazards or altered risks, so that their impact can be eliminated or minimized before product safety and quality are compromised *(4)*.

To adopt the HACCP concept for controlling hazards in food products efficiently, food manufacturers need to apply the prerequisite program, or Good Manufacturing Practices (GMP), to their process. The prerequisite program covers the controlling of premises, transportation and storage, equipment, personnel, sanitation, pest control and recall procedure *(8)*. A good prerequisite program can reduce the number of critical control points in the HACCP plan, which increases the efficiency of the HACCP program.

The specific aim of the present study was to define the hazards associated with manufacture of traditional dry sausages by gathering and evaluating information on processing conditions to define the critical control points (CCPs). The evaluation of each traditional sausage workshop was performed through use of a Good Manufacturing Practices and Good Hygienic Practices (GMP/GHP, HACCP) checklist according to an HACCP plan adapted for use by small producers.

Identification of the hazards and quantitative assessment of risks associated with dry sausages will provide traditional producers with validated control measurements and critical limits at process steps (Critical Control Points) for the manufacture of safe products.

MATERIALS AND METHODS

Checklist for auditing GHP/GMP, HACCP

In previous work, a study of the typology of traditional dry sausage producers in Catalonia (Spain) was undertaken. After statistical analysis consisting of a multifactorial (MFCA) and a cluster analysis (CA), four groups were obtained *(23)*. According to the size of each cluster, 2 or 3 workshops were selected from each of the four groups, in order to obtain ten representative workshops (C01 to C10) to be studied in this work.

Based on the United States Food and Drug Administration recommendations for HACCP plans *(2, 25)*, a questionnaire, with 105 questions, was established. It was administered by a direct interview with the owner of each workshop. This questionnaire consisted, basically, of two parts. The first part was related to the prerequisites for a self-control system based on the HACCP plan (building and facilities, sanitary facilities, equipment, hygiene and sanitation, production and process controls). The second part evaluated some critical points of the process and the efficiency of the hygienic program used in the workshop equipment (equipment, hygiene and sanitation, production and processing controls), protocols for microbial content evaluation, and measurements of temperature/humidity. Points were derived from the questionnaire, allowing the workshops to be ranked. The maximum possible total scores were 61 and 50 points for the first and second parts of the questionnaire, respectively. Workshops exhibiting scores of 30 points or more for each part were classified as "Sufficient" based on the criteria established, taking into account that not all the questions had the same importance.

Regarding microbiological criteria, two parameters were agreed upon: Hygienic markers (for surfaces) and pathogen content (for surfaces and final products).

Enterobacteriaceae was selected as the hygienic marker for environment and machines according to DOCE (2001) *(14)*. The established criteria in this work were as follows: (i) "ultraclean": $< 2 \times 10^2$ CFU/100 cm^2, (ii) "clean": 3-9 $\times$ 10^2 CFU/100 cm^2 and (iii) "not clean": $> 10^3$ CFU/100 cm^2.

The pathogens assessed were *Salmonella* spp., *Staphylococcus aureus, Listeria monocytogenes* and *Escherichia coli* verotoxigenic (VTEC). For safety evaluation of products, the criteria limits were established as follows *(17, 35)*: (i) *Salmonella* spp.: absence in 25 g of final product, (ii) *E. coli* (VTEC): absence in 25 g, (iii) *S. aureus*: < 500 CFU/g, (iv) *L. monocytogenes*: < 100 CFU/g.

Physical analyses

Temperature and relative humidity measurements were carried out using a Testostor 175-Data Logger (Testo GmbH & Co. Lenzkirch, Germany).

Sampling procedures

Environmental surfaces: Mincing, mixing and stuffing machines; knives; cutting tables; and walls of the cold storage room were selected for microbial analyses. A total of 500 cm^2 of each cleaned surface was sampled by use of a 40 $\times$ 40 cm cloth wet with neutralizing solution (Laboratoires Humeau, La Chapelle sur Edre, France). Buffered peptone water (BPW) (AES Laboratoires, Bruz, France) was the diluent solution. The initial environmental suspension (IES) was prepared by pummeling the swab (40 $\times$ 40 cm cloth) with 25 ml of diluent. Serial dilutions were

TABLE 1. Results (%) obtained in GMP/GHP checklist by the workshops on Part I: Buildings and Facilities

	Very Good	Yes/Good	No/Not Good
- Approval by competent authorities	0	100	0
- Water supply with treatment	0	100	0
- Open windows: fine mesh to keep out insects	0	100	0
- Sufficient ventilation to minimize odors/vapors/ prevent water condensation	0	100	0
- Lights in processing areas equipped with proper covers	0	100	0
- Materials used in the construction/easy cleaning	0	90	10
- Electric fly traps?	0	90	10
- Selection and separation of solid trash	0	90	10
- Waste treatment	0	90	10
- Walls and floor material's conservation	20	80	0
- No existence of cross paths	0	70	30
- Walls and floor hygiene level	20	70	10
- Existence of foot washing device	0	0	100

then prepared in BPW and plated onto the appropriate culture media as described below.

Meat products: Depending on the size of sausages, 3 to 4 dry sausages (approximately 500 g) of the same batch, without casings, were pooled and mixed, to obtain a representative meat sample. For *Enterobacteriaceae, S. aureus, L. monocytogenes, Salmonella* and *E. coli* VTEC determinations, a sample of 25 g was diluted in 225 ml of BPW and blended for 1 min in a stomacher (Model 400 Cooke Laboratories, Alexandria, VA, USA). For *L. monocytogenes* quantification, a meat sample of 20 g was diluted with 40 ml of BPW (1:3) and blended in a stomacher for 1 min. When necessary, a serial dilution was performed.

Microbial analysis

Spoilage flora and pathogens were enumerated by classical microbial procedures, using selective media. Presence and absence of *L. monocytogenes, Salmonella* and *E. coli* VTEC were also investigated by polymerase chain reaction PCR. The same selective culture media were used for both environmental and meat samples.

Enterobacteriaceae: Appropriate dilutions of meat and environmental samples were plated on Crystal Violet neutral Red Bile Glucose agar (VRBG) from Merck (Darmstad, Germany). The plates were incubated for 24 h at 37°C. After colony enumeration, biochemical confirmation was performed with an oxidase test (Merck, Darmstad, Germany). Only oxidase-negative colonies were taken into account for final enumeration.

S. aureus: Baird-Parker medium supplemented with RPF (bioMérieux, France) was used according to ISO 6888,2 (1999) *(6)*. Duplicates of 1 ml of appropriate dilutions were plated and incubated for 24–48 h at 37°C. Black colonies with a halo were enumerated as *S. aureus* and confirmed by PCR.

Listeria monocytogenes: To quantify after 1 h of resuscitation at room temperature, duplicates of 0.5 ml of meat and environmental sample dilutions were plated onto ready-to-use Aloa plates (AES Laboratoires, Bruz, France) validated by AFNOR *(12)* and incubated for 24–48 h at 37°C. Blue colonies with a white halo were enumerated as *L. monocytogenes* and confirmed by PCR. For presence/absence determination, 24-h pre-enriched cultures in BPW (2 ml) were subjected to a secondary enrichment for 48 h at 37°C on 18 ml of Fraser broth (Difco, Detroit, MI, USA) and confirmed by PCR.

Salmonella: A 24-h enrichment at 37°C was performed on IES and meat suspensions in BPW before plating. Semi-solid Rappaport Vassiliadis (MSRV) agar plates (Oxoid, Basingstoke, Hampshire, England) were spotted with three drops (approx. 100 µl) of the pre-enriched cultures and incubated for 20–22 h at 42°C. If a halo was visible around the inoculation spot, a loopful from the edge of the halo was streaked onto Brilliant Green Agar (BGA) (Difco, Detroit, MI, USA). Pink-red colonies with a red halo were confirmed by PCR.

E. coli VTEC: An enrichment of IES and meat samples in BPW for 24 h at 37°C was performed. The enriched culture was streaked onto McConkey agar (Difco, Detroit, MI, USA) and incubated for 24 h at 37°C. Red colonies with a bile-salt precipitate were confirmed by PCR. For presence/absence determination, an enrichment of IES and meat samples on Trypticase Soy Broth + novobiocin (Difco, Detroit, MI, USA) was carried out for 24 h at 37°C and confirmed by PCR.

PCR ANALYSIS

PCR pre-treatment

For PCR determination from sample, 2 ml of the enriched cultures were dissolved in 300 µl of 6% Chelex® 100 (BioRad), incubated at 56°C for 20 min, boiled for 8 min and cooled on ice. Samples were centrifugated at 14,000 rpm for 5 min and the supernatants used for PCR reaction.

TABLE 2. Results (%) obtained in GMP/GHP checklist by the workshops on Part I: Sanitary facilities

	Very Good	Yes/Good	No/Not Good
- Establishment in compliance of some Code of Hygiene Good Practices	0	100	0
- Hand-washing facilities adequate and sufficient	0	100	0
- Effective hand-cleaning/sanitizing preparations	0	100	0
- Presence of hand drying devices	0	100	0
- Presence of garbage cans	0	100	0
- Toilets	0	100	0
- Toilets, urinals and rest areas kept clean	30	70	0
- Pest Control: control plan conducted by a specialized enterprise	0	40	60
- Rest areas	0	30	70

TABLE 3. Results (%) obtained in GMP/GHP checklist by the workshops on Part I: Production and process controls

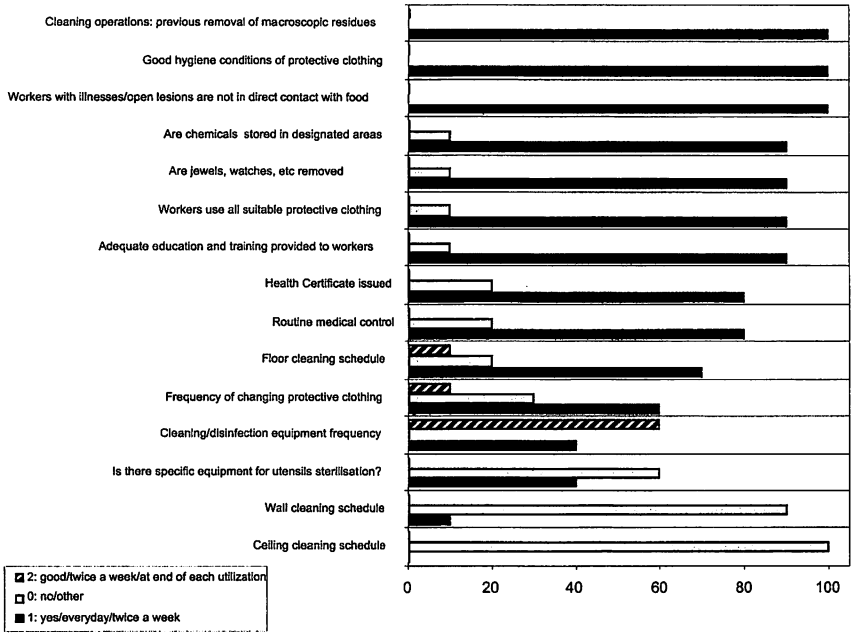
	Yes	No
- Are food additives stored in designated areas?	100	0
- Are raw materials and ingredients checked on their "best before" date?	90	10
- Are there specific demands on raw material characteristics?	90	10
- Is there a regular monitoring of the process's time and temperature?	90	10
- Is there an operative process control system?	70	30
- Is there handling of other meat species, apart from pork and beef?	60	40
- Is there a sampling analyses control plan?	60	40
- Are records kept on raw materials and other ingredients received?	50	50
- Is a record kept with this information?	50	50
- Is there some kind of documentation control?	50	50
Are relevant documents kept in a specific record?	40	60

For colony confirmation, the presumptive pathogen colonies were resuspended in 30 µl of sterile distilled water and 2–5 µl of this suspension was added to the PCR reaction mix.

For *L. monocytogenes, Salmonella* and *E. coli*, a PCR amplification protocol from Simon et al. *(37)*, Rahn et al. *(33)* and Abdulmawjood et al. *(1)*, respectively, and validated under the European Project FOOD-PCR *(15)*, was applied with the corresponding internal amplification controls. *S. aureus* confirmation was carried out using the PCR protocol developed by Martineau et al. *(29)*.

A 20 µl aliquot of each PCR product was subjected to 1.5% (w/v) agarose gel electrophoresis containing 0.1 µg/ml ethidium bromide (Sigma) for 45 min at 100 V. The amplicon visualization was performed by use of a UV transilluminator (Pharmacia, LKB).

FIGURE 1. Percentage of workshops fulfilling aspects of GMP/GHP HACCP checklist. Part I: Hygiene and Sanitation



RESULTS

Building and facilities

A high percentage of workshops were considered to be satisfactory (Table 1). All evaluated enterprises had approval from appropriate authorities. The facilities were easy to clean; walls and floors were in good or very good state; and the visual hygiene aspect was excellent (90%), although 30% contained areas where raw and finished products cross paths. Furthermore, in none of the facilities was a foot washing device used, although some of them had one.

Sanitary facilities

The majority of facilities had a good level of sanitation (Table 2). All of them operated in compliance with some code of good hygiene practices. They had enough wash-hand equipment, with cleaning or sanitizing products. Most (70%) kept toilet and urinal areas clean. Only 40% followed a pest control plan conducted by a specialized enterprise.

Personnel hygiene and sanitation

In general, these aspects achieved the highest points in the majority of facilities. Routine medical control; suitable protective clothing; education and training of workers; storage of chemicals in designated areas; frequency of cleaning of floors and walls; and personnel hygiene and sanitation achieved maximum points in more than 80% of facilities. However, the majority of facilities do not have a ceiling cleaning schedule and only 40% used specific sterilization equipment (Fig. 1).

Production and process control

All facilities stored food additives in designated areas. In 60%, pork was not the only raw meat processed. An efficacious process control system was followed by 70% of producers. In addition, a sampling analysis control plan (generally every month on final product and surfaces, using classical or rapid tests) was applied by 60%. On the other hand, the absence of a plan for calibration control by a specialized enterprise was noted in 70%, and lack of documentation control in 50%, of facilities (Table 3).

CCP check action

The beginning of the process was under control in all the facilities. Also, rules regarding the meat and batter temperature, the "best before" date and the storage temperature of ingredients were usually respected, although, in 80% of cases, temperature and relative humidity (RH) in reception areas were not adequate (temperature higher than 12°C and RH below 85%). On the other hand, recorded temperature of carcasss/trimmings storage and resting rooms was correct (< 7°C), although humidity values were below 85% in 90% of facilities studied. Such low humidity could cause extensive dehydratation of carcasses or trimmings before utilization. Finally, excessive time (more than 4 h) for the casing desalting process was recorded (60%), although the process was carried out at an appropriate temperature (< 7°C) (Fig. 2).

Hygienic quality of environment and products

Regarding the microbiology of the environmental samples, walls of cold rooms earned an "ultraclean" classification (90%), as indicated by the fact that the *Enterobacteriaceae* content was below established criteria, although *L. monocytogenes* was detected in one facility. The mixing machines were also classified as "ultraclean" in 70% of cases. Likewise, knives (50%), mincing machines (40%) and cutting tables (30%) presented *Enterobacteriaceae* content below the criterion (200 UFC/100 cm^2), although in some of them, *L. monocytogenes* was detected. The highest rate of *Enterobacteriaceae* ("not clean") as well as the presence of pathogens were observed in stuffing machines, which proved to be the most contaminated equipment, with 70% of facilities obtaining a "not clean" or a "clean" (plus pathogen) classification. Moreover, in 20% *L. monocytogenes* was detected. Regarding hygienic classification of the final products, even though pathogens were detected in some samples, all the facilities achieved the maximum value, 8 points, indicating tolerable levels of pathogens according to the established criteria (Fig. 3).

All ten facilities studied recorded a "sufficient" score, 30 or more points for each of the two parts, C04 having the highest total score (92 points). C07 and C05 achieved the lowest scores, with 68 and 70 points, respectively (Table 4). However, some aspects and critical points, should be improved, as has been described

DISCUSSION

In order to guarantee the safety of food products based on HACCP principles *(9, 16)* Spanish legislation mandated the implementation of a self-control system for food industries in 1993. Total responsibility lies with the enterprises. The role of the administration is to verify that the industries implement and maintain, effectively, an effective self-control system *(3)*.

FIGURE 2. Percentage of workshops fulfilling aspects of GMP/GHP HACCP checklist. Part II: Critical Control Points - Check Action

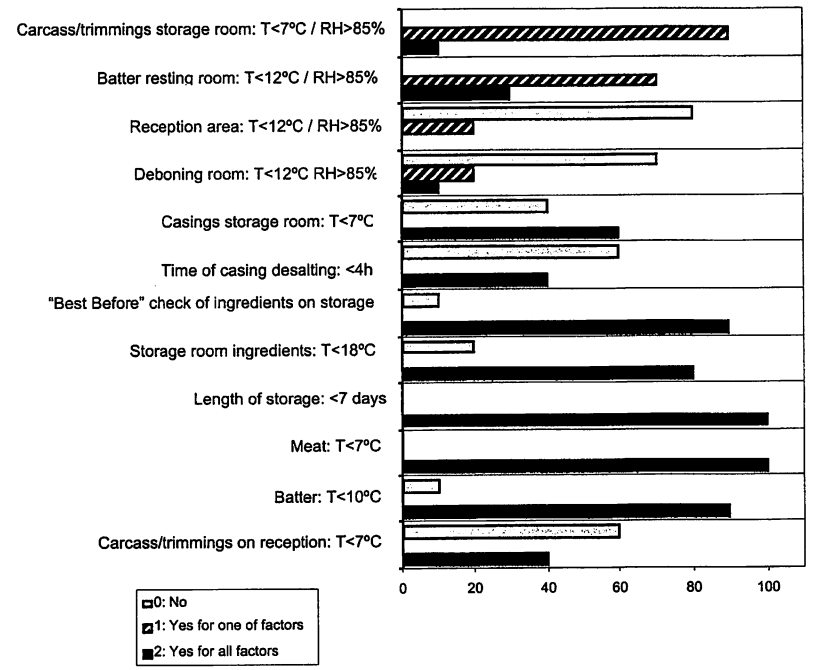
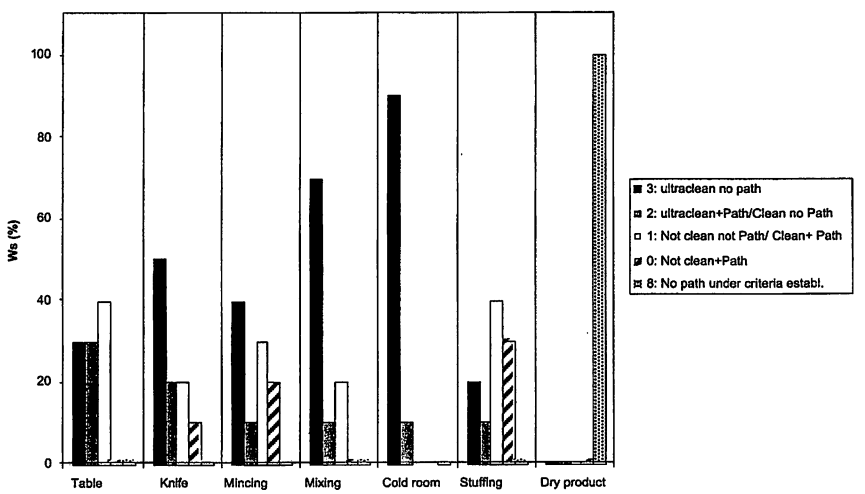


FIGURE 3. Percentage of workshops (Ws) fulfilling aspects of GMP/GHP HACCP checklist. Part II: Hygienic quality of environmental samples and products



A considerable number of "traditional factories" that exist in Spain have not standardized the manufacturing processes, and accordingly implementation of an HACCP system is more complicated. Moreover, the additional operating expenses caused by implementation of such a system impose economic difficulties on this type of enterprise.

The present work is intended to assist traditional workshops in Catalonia in applying the GMP/GHP checklist correctly. The checklist is well suited to this kind of factory, to reduce or minimize incorrect food handling practices. Such malpractices are considered, in the national epidemiological reports, to be the principal factors responsible for appearance of food poisoning outbreaks *(10, 13, 18, 24, 40)*. Based on the results obtained from this GMP/GHP checklist, we can assume that the traditional enterprises studied are able to implement a self-control system, and have thus fulfilled the majority of aspects treated. Even though the scores obtained exceeded the minimum pre-set marks (30 points), a strict adherence to Good Manufacturing Practices should be obligatory to guarantee a better safety margin for traditional dry sausages. For this reason, some aspects have to be subjected to corrective actions.

The "farm to fork" food strategy of the HACCP system involving all stages of the food chain has a clear purpose, which is to limit the hazards or risks associated with each step of the process. The purpose of this GMP/GHP checklist is also to minimize microbial contamination of the environment and raw materials and consequently to improve the safety of the final product. An item to be corrected in the Catalonian facility, is the relative lacks of specific equipment for sterilization of knives and other utensils. As stated elsewhere *(19)*, knives bore a lower contamination charge after ultraviolet (UV) light sterilization, compared to conventional disinfection procedures. Our work confirmed this observation; better hygiene marks were recorded for knives ("ultraclean" without pathogens) among facilities using UV sterilization equipment (30%). Likewise, the existence of cross paths, present in 30% of analyzed factories, is a risk to be taken into account. When building organization is not designed to avoid cross paths, the risk of microbial contamination of products with raw materials, personnel or instruments becomes likely. In this sense, foodborne pathogens such as *S. aureus*, which are ubiquitous and sometimes found on human/animal skin, could contaminate food through poor handling practices or existence of cross paths, or when temperature abuse occurs so as to lead to growth and enterotoxin formation *(5)*. Likewise, *L. monocytogenes*, widely distributed in the environment *(26, 27, 39)*, may enter abbatoirs with animals, but contamination of red meat often occurs because of the environment in which these foods are produced rather than from the animals themselves. Cross-contamination and recontamination may occur on transport vehicles and pieces of equipment that are difficult to dismantle and that thus continue to contaminate meat despite cleaning; they can also originate from the hands of workers and during cleaning operations *(5)*. Salvat et al. *(34)* reported that as many as 68% of environmental samples in a curing plant were positive for *L. monocytogenes* and that even after cleaning, 17% of the samples remained positive. In our study, *L. monocytogenes* was detected

TABLE 4. Scores obtained after GMP/GHP HACCP checklist application

	Workshops									
	C01	C02	C03	C04	C05	C06	C07	C08	C09	C10
Part I[1]	46	48	46	**50**	**38**	47	**38**	48	44	40
Part II[2]	39	38	37	**42**	**32**	37	**30**	38	42	39
Total score[3]	85	86	83	**92**	**70**	84	**68**	86	86	79

[1]Maximum 61 points; [2]: Maximum 50 points; [3]: Maximum 111 points

Criterion: Sufficient: 30 or more points for each of the two parts, Part I and Part II

in 10% of the 60 environmental samples after cleaning. Recent European investigations have reported 12–16% *Listeria*-positive isolation in industrial fermented meat products *(11, 21)*. In our study, *L. monocytogenes* was found in 1 out of 10 dry sausages analyzed, although in facilities where cross paths were found, no *L. monocytogenes* was detected, but *S. aureus* was counted in some instruments and in one final product. Nevertheless, in all facilities the pathogen concentration was below the pre-established acceptable limits agreed in this study.

According to HACCP principles, poor control of critical points, along with low hygiene status of the working environment and equipment, creates the probability of failures in the safety of final products. In fact, when facilities recorded pathogens on their final products, a direct or indirect relation with other tested parameters was observed: In three out of four facilities in which final products, carried pathogens, the temperature in reception and storage areas was high. Time-temperature abuse, reported as one of the major factors leading to food contamination during food preparation and storage, results in the survival, growth and production of toxins by pathogens *(30)*. In addition, in one facility, *S. aureus* was present in most of the pieces of equipment sampled. On the other hand, we can confirm the direct relation of final products carrying *S. aureus* with the presence of this pathogen in the initial periods of maturation (data not shown).

A real improvement of the hygienic status of facilities could be achieved if a higher percentage of traditional industries routinely implemented a sampling analysis control plan, testing microbial content of environmental and meat samples. Among enterprises studied, 60% carried out this practice.

Finally, records of control, monitoring, calibration and corrective actions should be kept, as these must be consulted during the process of verification *(4)*. Only 50% of enterprises studied implement this practice, a proportion that must be increased.

Little work has been reported on the implementation of this type of GMP/GHP checklist *(7, 32)*. However, studies about critical control point identification have been more frequently reviewed, maybe because it is the first step needed for the implementation of an HACCP plan *(30, 31, 36, 38)*. In various fermented products such as cheese, sausage or beverages the establishment of a manufacturing flow diagram is crucial for the determination of critical control points. Key points in the process flow diagram that constitute a likely source of contamination are relatively easy to establish: In general, as also recorded in the present work, reception and storage conditions of raw materials, weighing of ingredients, preparation of the batter (or liquor for beverages) and fermentation each constitute one of the generic critical control points for all food manufacturing processes *(20, 31)*. In consequence, they must be tested during the HACCP application.

Results obtained from this HACCP checklist application could help producers improve the hygienic quality of their facilities and products. The final consequence of this process should be an increase in traditional dry sausage quality. From a more global point of view, this work presents the basis for evaluating the hygienic quality of traditional food industries through the systematic application of this kind of checklist that will facilitate the implementation of an adequate HACCP plan.

ACKNOWLEDGMENTS

This work was funded by the European Project: Assessment and Improvement of Safety of Traditional Dry-Sausages from Producers to Consumers (Tradi-sausage –QLK1-CT2002-02240). The authors would like to acknowledge the collaboration of Isabelle Chevallier (ENITA, École National d'Ingenieurs des Travaux Agricoles de Clermont Ferrand, France) and Maria Joao Franqueza (Facultade de Medicina Veterinária de Lisboa, Portugal) in the construction of the HACCP questionnaire and the discussion of established criteria.

REFERENCES

1. Abdulmawjood, A., M. Bulte, N. Cook, S. Roth, H. Schonenbrucher, and J. Hoorfar. 2003. Toward an international standard for PCR-based detection of *Escherichia coli* O157. Part 1. Assay development and multi-center validation. J Microbiol. Methods 55(3):775–86.
2. Anonymous. 1995. Food and Drug Administration. PART 110 – Current good manufacturing practice in manufacturing, packing, or holding human food. Code of Federal Regulations. Title 21. Vol. 2. Washington, D.C.
3. Anonymous. 1996. Boletín Oficial del Estado. RD 2207/95, de 28 de diciembre, por el que se establece les normas de higiene relativas a los productos alimentarios. (BOE num 50, del 27 de febrero de 1996). Madrid, Spain.
4. Anonymous. 1996. Quality and safety assurance systems: Pre-requisites for quality control and quality assurance. Vol 10. *In* Concerted Action CT94–1456. Microbial Control on the Meat Industry. Bristol University. ISBN 0 86292 451 0. UK.
5. Anonymous. 1996. Bacterial pathogens on raw meat and their properties. Vol 7. *In* Concerted Action CT94-1456. Microbial Control in the Meat Industry: Bristol University. ISBN 0 86292 448 0. UK.

6. Anonymous. 1999. Microbiology of food and animal food stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 2: Technique using rabbit plasma fibrinogen agar medium. International Organization for Standardization ISO 6888,2. Geneva.
7. Anonymous. 1999. Guidelines for developing good manufacturing practices (GMP), standard operating procedures (SOPs) and environmental sampling/testing recommendations (ESTRs). Ready-to-eat products. Coordinated by National Meat Associations, April 1999. p. 1–21. Available at: http://www.nma-online.org/files/guifinal.pdf.
8. Anonymous. 1999. Food hygiene basic texts. Codex Alimentarius Commission. Codex Alimentarius: The Secretariat of the Joint FAO/WHO Food Standards Programme. Rome.
9. Anonymous. 2000. Boletín Oficial del Estado. RD 202/2000, de 11 de febrero, por el que se establecen las normas relativas a los manipuladores de alimentos (BOE núm 48, de 25 de febrero del 2000). Madrid, Spain.
10. Anonymous. 2000–2002. Boletines epidemiológicos y microbiol. semanales. Centro Nàc de Epidemiología. Instituto de Salud Carlos III, Madrid.
11. Anonymous. 2000. Études des risques liés à *Listeria monocytogenes*. Comission de l'Agence Française de Sécurité Sanitaire des Aliments. Maisons Alfort, France.
12. Anonymous. 2000. Validated Aloa plate for *L. monocytogenes* detection. AFNOR 10/3-09/00. Available at: http://www.aeslaboratoire.com/cgi-bin/produits_AES.pl?lang=fr&prodc=industrie&num3&prodnum=49.
13. Anonymous. 2000. European Commission. Health & Consumer Protection Directorate-General. Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health on FOOD-BORNE ZOONOSES (12 April 2000). *Campylobacter*. Brussels.
14. Anonymous. 2001. Diario Oficial de las Comunidades Europeas (DOCE). Decisión de la Comisión de 8/06/2001 por la que se establecen normas para los controles regulares de la higiene realizados por los explotadores de establecimientos. Diario Oficial de la Comunidades Europeas *(DOCE)* L165. p:48–53.
15. Anonymous. 2002. Validation of PCR protocols for *L. monocytogenes* and *Salmonella* spp. identification. European Project FOOD-PCR (QLK1-CT-1999-00226). Available at: http://www.pcr.dk/.
16. Anonymous. 2004. Sobre los controles efectuados para garantizar la verificación del cumplimiento de la legislación en materia de piensos y alimentos y la normativa sobre la salud animal y bienestar de los animales. Reglamento Europeo (CE) N 882/2004 del Parlamento Europeo y del Consejo. 29 de abril de 2004. L165/1.
17. Anonymous. 2004. Draft, commission of regulation on microbiological criteria for foodstuffs and food production. The Commission of the European Communities. SANCO/4198/2001, rev. 9. p. 1–35. Brussels.
18. Anonymous. 2004. Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo. Centro Nac de Epidemiología Boletín Epidemiológico Nacional. Sistema de Notificación de Microorganismos. Available at: (www.cne.isciii.es.)
19. Barandiaran Reus, M. 2001. Verificación de los sistemas APPC en mataderos y salas de despiece p. 449–459. *In* Martín & Macías (eds), Enciclopedia de la carne y de los productos cárnicos. Volumen I Chapter 23. ISBN: 84–85263–10–3. Plasencia, Cáceres – Spain.
20. Bryant, J., D. A. Brereton, and C. O. Gill. 2003. Implementation of a validated HACCP system for the control of microbiological contamination of pig carcasses at a small abatoir. Can. Vet. J. 4: 51–55.
21. Chasseignaux, E. 1999. Ecologie de *Listeria monocytogenes* dans les ateliers de transformation de viandes de volailles et de porcs. Université Claude Bernard. Lyon, France.
22. Demeyer, D. M., M. Raemaekers, A. Rizzo, A. Holck, A. De Smedt, B. Ten Brink, B. Hagen, C. Montel, E. Zanardi, E. Murbrekk, F. Leroy, F. Vandendriessche, K. Lorentsen, K. Venema, L. Sunesen, L. H. Stahnke, L. De Vuyst, R. Talon, R. Chizzolini, and S. Eerola. 2000. Control of bioflavor and safety in fermented sausages: first results of a European project. Food Res. Int. 33:171–180.
23. Fadda, S., T. Aymerich, M. Hugas, and M. Garriga. 2004. Tipología de pequeñas y medianas industrias productoras de embutidos curados de Cataluña. Eurocarne. 123:105–112.
24. Grillo Rodríguez, M. 1997. Higiene de los Alimentos, p. 405-433. *In* Martínez Navarro, F., J. M. Antó, P. L. Castellanos, M. Gili, P. Marset, V. Navarro, McGraw-Hill. (eds), Salud Pública. Interamericana, Madrid.
25. Jouve, J. L. 1994. La maîtrise de la sécurité et de la qualité des aliments par le système HACCP. *In* Multon, J. L. coordinnateur. La qualité des produits alimentaires, politique, incitations gestion et contrôle. p. 503–528, 750p. Tec & Doc Lavoisier. France.
26. Kathariou, S. 2000. Pathogenesis determinants of *Listeria monocytogenes*, p. 295–314. *In* Cary, J. W., J. Linz, and D. Bhatnagar (eds). Microbial foodborne diseases: mechanisms of pathogenesis and toxin synthesis. Technomic Publishing Co., Inc. Lancaster.
27. Kathariou, S. 2002. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. J. Food Prot. 65:1811–1829.
28. Lagrange, L., and J. Lelièvre. 1995. Propos sur la production fermière ou problématique de la production fermière, p. 67–72. *In* L. Lagrange (ed), Différenciation et qualité des produits alimentaires. ENITA, Clermont-Ferrand, France.
29. Martineau, F., F. J. Picard, P. H. Roy, M. Ouellette, and M. G. Bergeron. 1998. Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. J. Clin. Microbiol. 36:618–623.
30. Motarjemi, Y. 2002. Impact of small scale fermentation technology on food safety in developing countries. Int. J. Food Microbiol. 75:213–229.
31. Oranusi, S. U., V. J. Umoh, and J. K. P. Kwaga. 2003. Hazards and critical control points of Kunun-zaki, a non-alcoholic beverage in Northern Nigeria. Food Microbiol. 20:127–132.
32. Paukatong, K. V., and S. Kunawasen. 2001. The hazard analysis and critical control points (HACCP) generic model for the production of Thai

fermented pork sausage (Nham). Berl. Münch. Tierärztl. Wschr. 114:327–330.
33. Rahn, K., S. S. De Grandis, R. C. Clarke, S. A. Mc Ewen, J. E. Galán, G. Ginocchio, R. Curtiss, and C. L. Gyles. 1992. Amplification of an *invA* gene sequence of *Salmonella* Typhimurium by polymerase chain reaction as a specific method of detection of *Salmonella*. Mol. Cell. Probe. 6:271–279.
34. Salvat, G, M. T. Tonquin, Y. Michel, and P. Colin. 1995. Control of *L. monocytogenes* in the delicatessen industries: the lessons of a listeriosis outbreak in France. Int. J. Food Microbiol. 25:75–81.
35. Schalch, B., and H. Beck. 2001. National legislation, guidelines and standards governing microbiology – European Union. p. 1561–1564. *In* Robinson, R.K., C.A. Batt, and P.D. Patel (eds). Encyclopedia of Food Microbiology, vol. 3. Academic Press, London.
36. Silva, I. M. M., R. C. C. Almeida, M. A. O. Alves, and P. F. Almeida. 2003. Occurrence of *Listeria* spp. in critical control points and the environment of Minas Frescal cheese processing. Food Microbiol. 81:241–248.
37. Simon, C. M., D. I. Gray, and N. Cook. 1996. DNA extraction and PCR methods for the detection of *Listeria monocytogenes* in cold-smoked salmon. Appl. Environ. Microbiol. 62: 822–824.
38. van den Elzen, A. M., and J. M. Snijders. 1994. Identification of critical points in meat production lines regarding the introduction of *Listeria monocytogenes*. Vet. Q. 15:143–145.
39. Vazquez Boland, J. A., M. Kuhn, P. Berche, T. Chakraborty, G. Domínguez- Bernal, W. Goebel, B. González–Zorn, J. Wehland, and J. Kreft. 2001. *Listeria* pathogenicity and molecular virulence determinants. Clin. Microbiol. Rev. 14:584–640.
40. Yáñez Ortega, J. L., I. Carramiñana Martínez, and M. Bayona Ponte. 2001. Outbreak by *Salmonella* Enteritidis in a residential home for elderly people. Rev. Esp. Salud Pública. 75(1):81–88.