Destruction of *Trichinella spiralis spiralis* During the Preparation of the "Dry Cured" Pork Products Proscuitto, Proscuittini and Genoa Salami

H.J. Smith, S. Messier and F. Tittiger

ABSTRACT

Genoa salami, proscuittini and proscuitto were prepared from pork carcasses that were heavily infected experimentally with *Trichinella spiralis spiralis*. Genoa salami was prepared with salt concentrations of 2.0%, 2.75% and 3.3%. Proscuitto was prepared by two procedures approved by Agriculture Canada. At various times postpreparation, samples of the various cured products were taken and examined by pepsin digestion and rat bioassay for the presence of viable trichinae. Water activity and pH of the cured meat were also determined.

Curing of the various products was shown to destroy the *Trichinella* larvae. Pepsin digestion revealed that larvae progressively became loosely coiled, uncoiled and more subject to digestion (ghost larvae) during the curing process. Rat bioassay revealed the presence of viable trichinae in the proscuitto prepared using a sodium chloride salt mixture at day 34 but not at day 48 postpreparation. All other bioassays carried out on Genoa salami between 13 and 42 days postpreparation, on proscuittini between days 27 and 69 and on proscuitto between days 34 and 69 were negative for viable trichinae.

Under the conditions of this study, preparing Genoa salami with salt concentrations as low as 2% did not appear to affect the destruction of *Trichinella* larvae.

RÉSUMÉ

Cette expérience consistait à préparer du salami Genoa, du proscuittini et du proscuitto, à partir de carcasses de porcs préalablement soumis à une infection expérimentale avec environ 16 000 larves de *Trichinella spiralis spiralis*. La préparation du salami Genoa se fit avec respectivement 2%, 2,75% et 3,3% de sel et celle du proscuitto, selon deux procédés approuvés par Agriculture Canada. À divers intervalles ultérieurs à la préparation des produits précités, on en préleva des échantillons pour vérifier la présence de trichines viables, par la digestion peptique et la consommation par des rats. On détermina aussi l'activité aqueuse et le pH des dits produits.

Leur salaison entraîna la destruction des larves de trichines. La digestion peptique démontra que les larves perdaient graduellement leur aspect spiralé et devenaient plus sujettes à la digestion, au cours de la salaison. Le test de consommation par des rats démontra la présence de larves viables de trichines, dans le proscuitto préparé avec un mélange de sels, au bout de 34 jours, mais non au dbout de 48. Tous les autres tests de consommation par des rats, réalisés de 13 à 42 jours après la préparation du salami Genoa, de 27 à 69 jours après celle du proscuittini et 34 à 69 jours après celle du proscuitto, ne démontrèrent pas de larves de trichines viables.

Dans les conditions de cette expérience, la préparation du salami Genoa avec aussi peu que 2% de sel ne sembla pas affecter la destruction des larves de trichines.

INTRODUCTION

Traditional procedures for dry curing were established on an empirical basis. It has been shown experimentally that certain levels of salt in combination with drying for minimum periods at specified temperatures effectively destroy *Trichinella spiralis spiralis* larvae in pork products (1). More recently it was established that the effect of dehydration was decisive for the destruction of trichinae and bacterial pathogens (2).

New processing methods for cured products, many of them with a lower salt concentration than the required $> 3.3\%$ salt that the present Canadian regulations stipulate for dry cured products (3) are being proposed by industry. The effect of active water (aW) content and pH has been studied for a number of pathogens, but data are not available for various salt concentrations and curing schedules. The present study was undertaken to determine the maximal survival time of *Trichinella spiralis spiralis* larvae during the preparation of proscuitto (Italian ham), proscuittini (pork butt) and Genoa salami with varying salt concentrations, to correlate survival

Agriculture Canada, Health of Animals Laboratory, P.O. Box 1410, Sackville, New Brunswick E0A 3C0 (Smith), Agriculture Canada, Laboratoire d'hygiène vétérinaire, 3400 boul. Casavant, Saint-Hyacinthe, Québec J2S 8E3 (Messier), and Agriculture Canada, Meat Hygiene Division, Halldon House, 2255 Carling Avenue, Ottawa, Ontario K1A 0Y9 (Tittiger).

Submitted February 24, 1988.

of trichinae with aW and pH changes during the fermentation and drying periods and to assess the usefulness of aW and pH for evaluating the safety of dry cured pork products.

MATERIALS AND PRODUCTS

EXPERIMENTAL ANIMALS

Fifteen pigs weighing 62-65 kg were purchased from a commercial swine producer. Wistar laboratory rats for bioassay were purchased from a commercial animal breeder. Pigs and rats were maintained on commercially prepared feed. The Canadian Council on Animal Care guidelines outlined in a "Guide to the Care and Use of Experimental Animals, Volume I" were followed.

EXPERIMENTAL INFECTIONS

Each pig was given an estimated 16,000 *Trichinella spiralis spiralis* infective larvae by drenching. The *T. spiralis spiralis* isolate was originally recovered from a pig in Nova Scotia and had been maintained in Wistar rats since 1974. Sera were collected from all pigs on days 0, 19, 22, 26 and 33 postinfection and examined by enzyme linked immunosorbent assay (ELISA) for the presence of anti-*Trichinella* antibodies. The pigs were slaughtered 48 days postinfection. Between 200 g and 300 g of musculature including tongue, masseter, intercostal, shoulder, psoas and abdominal muscles were examined from each pig to determine the magnitude of the infections established. The remainder of each carcass was used in the preparation of proscuitto, proscuittini and Genoa salami.

PREPARATION OF PORK PRODUCTS

Genoa salami was prepared by grinding boneless pork through a 26 mm plate. Three batches were prepared containing salt (NaCl) levels of 2.0%, 2.75% and 3.3%. In addition, the mixture contained the following ingredients: sodium nitrate ($NaNO_3$) and sodium nitrite ($NaNO_2$), 150 ppm each; 1% spice mix (black pepper, nutmeg, red pepper, garlic); 0.5% dextrose and 0.5% red wine. The mixture was stuffed into 80 mm collagen casings and dried at 7°C and 80% relative humidity for three months. Starter cultures or elevated fermentation temperatures were not used.

Proscuittini was prepared by adding 4.5% NaCl, 150 ppm $NaNO_2$, 200 ppm $NaNO_3$, 1% erythorbate and 1% spices. Pork butts were tumbled with the curing mixture and then held at 2 to 3°C for 22 days. Then they were stuffed into natural beef casings (15 to 18 cm diameter) and hung to dry at 7°C and 80% relative humidity for two to three months.

Proscuitto type I was prepared as follows: hams, 7.5 to 8.5 kg in weight, were deboned and cured using a salt mixture containing 4.5% NaCl, 150 ppm $NaNO_2$, 200 ppm $NaNO_3$, 0.5% erythorbate and 0.5% spices in relation to the weight of the hams and were individually vacuum packed and stored at 7°C for 21 days. They were then removed from the cryovac bags, pressed into molds and hung for five days at 37.8°C. Hams were finally dried at 7.2°C and 45% relative humidity for four months. Upon completion of drying, the hams were washed and wrapped in aluminum foil paper.

Proscuitto type II was prepared from hams which were divided into two groups, those ≤ 7.7 kg and those > 7.7 kg in weight. Hams were first rubbed vigorously with a moistened NaCl/pepper mixture to facilitate penetration, followed by a dry NaCl mixture to a 3% salt concentration and then held for four days at 2 to 5°C and 75 to 95% relative humidity. Hams were then machine massaged further with 2% additional salt and subjected to a second curing at 2 to 5°C and 65 to 85% relative humidity for seven or ten days depending on the weight of the individual ham. The hams were machine brushed, massaged again and held at 2 to 5°C for 40 days followed by washing in an automatic washer and drying for 15 days at room temperature of 22°C. Salt and pepper were then applied to the surface of the hams. Hams were held in curing rooms at 15 to 18°C for four to six months to give a total curing time of six to eight months. No other curing aids except salt and pepper were used with this method of preparation.

LABORATORY PROCEDURES

Pepsin-digestion of fresh musculature and cured pork products was carried out using a 1% pepsin-1% HCl digestion mixture as described previously (4).

Rat bioassays were carried out either by feeding cured pork products or administering larvae by drenching. Rats were killed 29 to 33 days postinfection, skinned and eviscerated. The whole carcass was put through a meat grinder, digested and examined for the presence of *Trichinella* larvae.

Water activity (aW) and pH measurements were determined on core samples of the cured pork products as outlined by Messier *et al* (5).

Serological testing of sera for the presence of anti-*Trichinella* antibodies was carried out as previously described using the ELISA and a *T. spiralis spiralis* excretory-secretory antigen (4).

EXPERIMENTAL TRIALS

Genoa salami: 50 g of meat at each salt concentration were digested on days 13, 15, 19, 26, 27, 29, 34, 42 and 76 postpreparation. Larvae recovered from each salami preparation on days 13, 27, 29, 34 and 42 postpreparation were administered to rats by drenching. Ten gram samples of pooled salami of each salt concentration collected on day 11 postpreparation were also fed to individual rats.

Proscuittini: 50 g of pooled tissue from five randomly selected pork butts were digested on days 27, 34, 42 and 48 postpreparation. Larvae recovered on days 27 and 34 postpreparation were given to rats by drenching. On days 55, 62 and 69 postpreparation, 50 g samples from five randomly selected pork butts were individually digested and larvae from each were given to a rat.

Proscuitto: 50 g pooled samples of each type of proscuitto were digested on days 34, 42 and 48 postpreparation. Larvae recovered on day 34 postpreparation from each type were given to a rat. Ten grams of proscuitto of each type were also fed to a rat. On days 55, 62, 69, 77, 84, 90 and 96 postpreparation, 50 g samples from five randomly selected proscuitto I and proscuitto II hams were individually digested. Larvae recovered on days 55, 62 and 69 days postpreparation were administered to rats.

RESULTS

INFECTIONS ESTABLISHED

At slaughter infections were found in all pigs ranging from 38.6 to 460.7 *Trichinella* larvae per gram (1/g) of musculature. Mean infection for the 15 pigs was 159 1/g of muscle.

TABLE I. Active Water and pH Determinations for Genoa Salami with Salt Concentration of 2.0%, 2.75% and 3.3% from 1 to 74 Days Postpreparation

Days Postpreparation	% Salt	aW[a]	pH
1	2.0	0.975	4.99
	2.75	0.978	5.48
	3.3	0.930	5.51
4	2.0	0.975	5.66
	2.75	0.970	5.77
	3.3	0.955	5.79
11	2.0	0.955	4.84
	2.75	0.950	4.86
	3.3	0.930	4.87
13	2.0	0.955	4.84
	2.75	0.950	4.86
	3.3	0.930	4.87
18	2.0	0.938	4.92
	2.75	0.931	4.89
	3.3	0.939	4.89
20	2.0	0.960	4.84
	2.75	0.945	4.70
	3.3	0.925	4.87
25	2.0	0.938	4.77
	2.75	0.935	4.78
	3.3	0.925	5.76
27	2.0	0.952	4.71
	2.75	0.937	4.70
	3.3	0.922	4.77
32	2.0	0.930	4.90
	2.75	0.938	4.83
	3.3	0.926	4.79
40	2.0	0.929	4.84
	2.75	0.949	4.81
	3.3	0.902	4.79
74	2.0	0.880	4.81
	2.75	0.870	4.92
	3.3	0.870	4.82

[a]Active water

SEROLOGICAL FINDINGS

All pigs were negative for anti-*Trichinella* antibodies prior to experimental infection. By day 26 postinfection all pigs except one had seroconverted. One week later this pig had also seroconverted.

ACTIVE WATER AND pH DETERMINATIONS

Results for the three salt concentrations of salami and proscuittini and proscuitto types 1 and 2 are given in Tables I and II respectively.

PEPSIN DIGESTION

Genoa salami: With a salt concentration of 2.0%, up to 3% of larvae were still tightly coiled on day 27 postpreparation. The remainder of the larvae were loosely coiled or uncoiled. With a concentration of 2.75% salt, tightly coiled larvae were observed only up to day 19 postinfection. With a concentration of 3.3%, only loosely coiled or uncoiled larvae were recovered from all samples tested. By day 42 postpreparation only uncoiled or ghost larvae were recovered from salami at all salt concentrations.

Proscuittini: On days 27 and 34 postpreparation, up to 70% larvae recovered were tightly coiled. By day 56 postpreparation larvae recovered in all digestions were uncoiled or ghost larvae.

Proscuitto types I and II: On days 34 to 48 postpreparation, from 20 to 60% of larvae recovered were coiled. The remainder were loosely coiled or uncoiled. From day 55, all larvae were either loosely coiled, uncoiled or ghost larvae with a greater proportion of ghost larvae as curing progressed.

RAT BIOASSAY

Genoa salami: Trichinella infections were not established in any of the 18 rats given larvae recovered from salami with salt concentrations of 2.0%, 2.75% and 3.3% and examined between 13 and 42 days postpreparation.

Proscuittini: Trichinella infections were not established in any of the 16 rats administered larvae recovered from pork butts 27 to 69 days postpreparation.

Proscuitto: Trichinella infections were not established in any of the 17 rats administered larvae recovered from type I proscuitto 34 to 69 days postpreparation. A mean infection of 22.4 *Trichinella spiralis spiralis* 1/g was established in the rat fed larvae from a pooled sample of type II proscuitto on day 34 postpreparation. No infections were established in the remaining 16 rats fed type II proscuitto 55 to 69 days postpreparation.

DISCUSSION

The results of the trials demonstrate that the curing processes used in the preparation of Genoa salami, proscuittini and proscuitto effectively destroy *Trichinella spiralis spiralis* larvae. Pepsin digestion of fresh musculature at slaughter and enzyme linked immunosorbent assay of sera collected pre and postinfection confirmed that all pigs used in this study were infected with trichinosis. Seroconversion in most pigs occurred during the fourth week postinfection which agrees with previous studies (6).

Transmission of *T. spiralis spiralis* larvae was demonstrated only for type II proscuitto on day 34 postpreparation. Rat bioassays of Genoa salami with various salt concentrations, proscuittini and all other proscuitto samples at various time periods postpreparation showed that the trichinae had been destroyed during the curing process. It should be pointed out that rat bioassay has been used for many years to assess viability of *Trichinella spiralis spiralis* in pork products (7,8). Furthermore, rats were susceptible to the *Trichinella* isolate used as shown by the fact that it had been maintained in rats since 1974.

TABLE II. Active Water and pH Determinations for Proscuittini and Proscuitto from 25 to 95 Days Postpreparation

Days Postpreparation	Proscuittini		Proscuitto type I		Proscuitto type II	
	aW[a]	pH	aW	pH	aW	pH
25	0.925	5.71	-	-	-	-
32	0.940	5.67	0.919	5.71	0.922	5.51
40	0.900	5.58	0.920	5.60	0.920	5.60
46	0.890	5.62	0.914	5.50	0.930	5.54
53	0.860	5.80	0.920	5.70	0.895	5.80
60	0.875	5.81	0.900	5.75	0.920	5.81
68	0.895	5.80	0.930	5.40	0.926	5.70
74	0.835	5.90	0.890	5.60	0.915	5.50
81	0.855	5.80	0.920	5.45	0.920	5.62
88	0.875	5.92	0.890	5.68	0.922	5.68
95	-	-	0.910	5.77	0.920	5.83

[a]Active water

Differences in the infectivity of type I and type II proscuitto hams 34 days postpreparation may be related to curing salt mixtures used. Type I proscuitto was cured with a NaCl, sodium erythorbate, $NaNO_3$, $NaNO_2$ salt mixture, while only NaCl was used to cure the type II proscuitto. Allen and Goldberg (9) observed that larvae in sausage treated with a curing mixture containing NaCl and $NaNO_3$ appeared to lose their infectivity sooner than those in sausage containing only NaCl.

Pepsin digestion of the cured pork products also indicated destruction of trichinae during the curing process. Ransom *et al* (10) pointed out that dead larvae are uncoiled. In this study, the proportion of larvae that became loosely coiled, uncoiled or ghost larvae was progressively greater as curing took place.

In 1920, Ransom *et al* (10) showed that the effectiveness of the curing process in destroying *Trichinella* larvae is dependent upon salt concentration, temperature and time. In 1970, Zimmermann (1) showed that the drying temperature was the critical factor in killing trichinae. Lotzsch *et al* (2) found aW values (measure of biological available water) of 0.942 in sausages and hams in which the viability of trichinae had been destroyed. They suggested that once the aW value was reduced to 0.90, one could assume *Trichinella* larvae were no longer infective. The aW values of the cured salami and hams of this study were consistent with those observed by Lotzsch *et al* (2). Reduced salt concentration of 2.0% in salami with appropriate drying times appear sufficient to achieve the required degree of dryness to destroy viability of *T. spiralis spiralis* larvae. Measuring pH and aW values in dry cured products offers a quick and simple method to evaluate the safety of products where formulation and method of preparation are not known. Water activity values below 0.920 combined with a pH of less than 5.3 appear to be safe cut off points (2). Greater precision as to actual death point of *T. spiralis spiralis* as well as other pathogens, notably *Salmonella* species and *Listeria monocytogenes* would be desirable.

ACKNOWLEDGMENTS

The authors acknowledge with gratitude the participation and cooperation of H. St. Jean et Fils (1976) Inc., Saint-Hyacinthe, Quebec; J. Poulin, A. Bourgeois, Le Produits d'Alimentation Bologna Compagnie Ltee, Montreal, Quebec and Primo Division, Primo Food Ltd., Laval, Quebec in the preparation of the various pork products.

The authors are grateful to Mr. K.E. Snowdon and Ms. J. Gelinas for their technical assistance.

REFERENCES

1. **ZIMMERMAN WJ.** Salt cure and drying time and temperature on viability of *Trichinella spiralis* in dry cured hams. J Food Sci 1971; 36: 58-65.
2. **LOTZSCH R, RODEL W, BOHN H, MEYER W, PONERT H.** Untersuchungen uber die Lebensfahigkeit von *Trichinella spiralis* in Rohurirsten in Abhangigkeit von der Wasserahtivitat. Die Fleischwistschuaft 1974; 7: 1203-1208.
3. **ANONYMOUS.** Meat Hygiene Manual. Part A, Chapter 4, Annex A and B. Meat Hygiene Division, Food Production and Inspection Branch, Canada Agriculture, Ottawa, 1983.
4. **SMITH HJ.** Evaluation of the ELISA for the serological diagnosis of trichinosis in Canadian swine. Can J Vet Res 1987; 51: 194-197.
5. **MESSIER S, SMITH HJ, TITTIGER F.** Survival of *Salmonella typhimurium* and *Staphylococcus aureus* in Genoa salami of varying salt concentration. Can J Vet Res 1989; 53: 84-86.
6. **SMITH HJ.** Comparison of pepsin-digestion and enzyme-linked immunosorbent assay for the diagnosis of trichinosis in swine. Can J Vet Res 1988; 52: 63-66.
7. **RANSOM BH.** Effects of refrigeration upon the larvae of *Trichinella spiralis*. J Agric Res 1949; 5: 819-854.
8. **SMITH HJ.** An evaluation of low temperature sterilization of trichinae infected pork. Can J Comp Med 1975; 39: 316-320.
9. **ALLAN RW, GOLDBERG A.** The effect of various salt concentrations on encysted *Trichinella spiralis* larvae. Am J Vet Res 1962; 23: 580-586.
10. **RANSOM BH, SCHWARTZ B, RAFFENSPERGER HB.** Effects of pork curing processes on trichinae. Bull No. 880, USDA, Washington, D.C., 1920.