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Residual Viruses in Pork Products

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Partly cooked canned hams and dried pepperoni and salami sausages were prepared from the carcasses of pigs infected with African swine fever virus and pigs infected with hog cholera virus. Virus was not recovered from the partly cooked canned hams; however, virus was recovered in the hams before heating in both instances. Both African swine fever virus and hog cholera virus were recovered from the dried salami and pepperoni sausages, but not after the required curing period.

Animals in certain stages of infection may escape detection by the usual ante- or post-mortem inspection at abattoirs. Meat from such animals may inadvertently be included in commercial products. Such contamination may be called primary contamination as compared with secondary contamination, which occurs during processing, storage, or distribution of the product (3). In primary contamination, one considers animal products because animals become infected with a great variety of viruses, and some of these viruses inevitably occur in food products prepared from such animals. Some of these viruses, such as those of foot-and-mouth disease (FMD), hog cholera (HC), African swine fever (ASF), and Newcastle disease, although of great economic importance, are of little or no concern to human health (3).

Investigations of outbreaks of swine vesicular disease (SVD) have incriminated feeding of garbage contaminated with SVD virus (SVDV)-infected meat scraps. Infectivity is reduced only slightly or not at all in cold storage, so uncooked pork and pork products could remain a hazard indefinitely (2).

FMD virus (FMDV), however, will survive only if the pH remains above 6.2. Bacon carcasses treated by wet or dry salting and untreated bone marrow sometimes contain virulent virus for at least 42 days. The inactivation of FMDV in the muscles of a carcass is generally brought about by the production of lactic acid. Quick freezing of beef stops acid formation, and FMDV has been recovered as long as the meat is kept frozen. However, thawing of this quick-frozen meat allows acid formation to resume at an accelerated rate that rapidly produces an environment unsuitable for FMDV survival. Acid formation is not as great in lymph nodes and blood as in muscle, and the virus is likely to survive longer there even with delay in freezing and thawing. This prolonged survival is true even in lymph nodes and residual blood vessels of carcasses in which rigor mortis has been complete (5-7).

Pork products imported into the United States that might be potential hazards for the spread of such diseases as SVD and FMD include partly cooked canned hams, dry salami sausage, dry pepperoni sausage, and processed intestinal casings. Cured and dried products such as salami and pepperoni sausage originating from countries where SVD is present are no longer permitted entry into the U.S. except for further processing by heating to an internal temperature of 74.5°C (166°F) (12). Perishable canned meat products, e.g., partly cooked hams, have been found to be free of SVDV and FMDV when the internal temperature of processing reaches 69°C (156°F) (6).

The following experiments were performed to determine whether the viruses of ASF and HC could remain viable in such products.

MATERIALS AND METHODS

Virus. The ASF virus (ASFV) used was from an outbreak in Portugal in 1960 (10). It was a 1:10 suspension of spleen and blood from the fifth laboratory passage in domestic pigs and was designated as ASF L'60, batch 5. It was titrated by the hemadsorption reaction (9) in swine buffy coat cultures prepared as described elsewhere (8), and the titers were expressed as the log of 50% hemadsorption (Had_{50}) per milliliter.

The HC virus (HCV) used was NADL VIII PIADC 1, 1:10 suspension of spleen and blood. It had a titer of $10^{5.3}$ plaque-forming units per ml.

Source of meat. Four cross-bred Tamworth swine weighing 125 pounds (56.8 kg) each were inoculated with ASFV (5 ml intravenously and 5 ml intramuscularly [i.m.]). All swine had fever ranging from 40.4°C (104.8°F) to 41.6°C (106.8°F) in 24 h. Swine were slaughtered 48 h after inoculation, and the carcasses were hung at 4°C (39°F) for 48 h.

Two cross-bred Tamworth swine weighing approx-

imately 125 pounds each were inoculated i.m. with HCV. Both swine were killed on day 5 at peak of temperature, and the carcasses were hung at 4°C in a cold room for 48 h. The pH of the meat for both groups was 5.6.

Product preparation. All meat, including hams, was deboned, and excess fat was removed. Fifty pounds (22.7 kg) of meat was ground with a 3/16-inch (4.8-mm) plate for preparation of salami sausage, and 50 pounds of meat was ground with a 1/8-inch (3.2-mm) plate for preparation of pepperoni sausage.

(i) Hams. Hams weighing about 2 pounds (0.80 kg) each were injected with a 16% by weight brine solution and submerged in the same solution for 24 h at 4°C. The hams were then tightly packed in cans, and the cans were sealed. Some of the canned hams were submerged in a 37.8°C (100°F) water bath, and a similar number were kept as unheated controls at 4°C. The internal temperature of the ham was 2.5°C (37°F) at the start of the experiment. The bath temperature was slowly increased so that in about 3.5 h, the internal temperature of the ham was 69°C. The hams (Perishable Keep Under Refrigeration) were then placed in a cooling bath at 4°C for about 2 h and placed in a cold room at the same temperature.

(ii) Salami sausage. The ground meat was thoroughly mixed with the recommended amounts of salt, sugar, dextrose, white pepper, garlic, sodium nitrite, and sodium nitrate (4, 11). After a 48-h curing period at about 4°C, a lactic acid starter culture (Lactacel, Merck and Co., Inc.) was thoroughly mixed with the meat. The pH at this time was 5.5. The mixed meat was then packed into 1.5-inch (37.5-mm) diameter casings and kept in an environmental control chamber at 20°C (68°F) and a relative humidity of 68% for 48 h. The salami sausages were then placed in the smoking chamber and exposed to smoke produced from hickory chips for 12 h at 32°C (89.6°F) and a relative humidity of 80% and for an additional 12 h at 49°C (120°F) and a relative humidity of 58%.

The sausages were then removed from the smoking chamber, washed with warm water to remove surface juices and fat, and placed in a drying room at 11.7°C (52°F) and 72% relative humidity for not less than 25 days. The pH of the meat was determined at the time of grinding, after mixing, and in the finished product.

(iii) Pepperoni sausage. The ground meat was thoroughly mixed with recommended amounts of salt, sugar, dextrose, sodium nitrate, sodium nitrite, cayenne pepper, pimento, aniseed, garlic powder, and pepperoni pepper and was kept for 48 h at 4°C (4, 11). Lactic acid starter culture (Lactacel) was thoroughly mixed with the meat. The mixture was packed into casings of 1-inch (25-mm) diameter and kept in an environmental control chamber at 20°C and a relative humidity of 60% for 48 h. The pepperoni sausages were then placed in the smoking chamber for 8 h at a temperature of 32.2 to 34.4°C (90 to 94°F) and a relative humidity of 85%. After cooling, the sausage was washed with warm water and placed in a drying room at 11.7°C and 72% relative humidity for not less than 16 days.

Sampling of products. Triplicate samples of approximately 1 g (0.035 ounce) of each of the following products were examined for residual ASFV and HCV before and after smoking and at intervals as shown in Tables 1, 2, and 3: whole (unground) meat samples from the carcasses, ground meat samples, brined ham, heated ham, and pepperoni and salami sausages.

Virus assay. The samples for ASFV detection were ground with alundum with a mortar and pestle and suspended in Hanks balanced salt solution with lactalbumin hydrolysate culture fluid to give a 20% suspension. The suspension was thoroughly mixed in a Vortex mixer and then centrifuged for 5 min at 1,000 × *g*. The supernatant fluid was diluted serially in lactalbumin hydrolysate and assayed in swine buffy coat cultures; four cultures were used per dilution. The cultures were examined for hemadsorption at 24, 48, and 72 h. In samples in which negative or uncertain reactions were obtained at 72 h, fluids from cultures inoculated with the 10^{-1} and 10^{-2} dilutions were subpassaged to fresh buffy coat cultures. All cultures were retained for a final reading at 7 or 8 days postinoculation (DPI).

A 20% (wt/vol) suspension of each HC sample was made in Eagle minimum essential medium containing antibiotics (100 units of penicillin, 100 μg of strepto-

TABLE 1. *Recovery of ASFV in meat samples by animal inoculation*

Sample	Days after slaughter	Virus recovery (Had_{50}/g)
Whole meat	2	$10^{3.25}$–$10^{3.75}$
Ground meat	2	$10^{3.25}$–$10^{3.75}$
Salami + ingredients + starter	3	$10^{2.0}$–$10^{2.5}$
Pepperoni + ingredients + starter	3	$10^{3.0}$–$10^{3.25}$
Ham brined	2	$10^{2.5}$–$10^{3.75}$
Ham heated	5	Negative
Pepperoni sausage	8	$10^{2.75}$–10^{3}
Salami sausage	9	10^{-1}
Pepperoni sausage	30	Negative
Salami sausage	30	Negative

TABLE 2. *Recovery of HCV in meat samples by animal inoculation*

Sample	Days after slaughter	Response of inoculated animals[a]	
		Pig 1	Pig 2
Whole meat	1	+	+
Ham brined	2	+	+
Pepperoni + ingredients + starter	2	+	+
Salami + ingredients + starter	2	+	+
Ham heated	5	−	−
Pepperoni sausage	22	+	+
Salami sausage	21	+	−

[a] Two pigs with each sample. +, Infected (developed signs and lesions of HC); −, noninfected (remained normal).

TABLE 3. *Results of fluorescent antibody cell culture technique in pork products from swine infected with HCV*

Sample	Days after swine slaughtering	Virus isolation in PK-15 cell cultures[a]	Virus isolation after passage in primary swine cell cultures[a]	Titer of HCV (PFU[b]/g)
Whole meat from pig killed 5 DPI	0	+	NT[c]	$10^{1.5}$–$10^{1.87}$
Pepperoni + ingredients + starter	2	−	+	1–10
Salami + ingredients + starter	2	−	+	1–10
Ham brined	4	−	+	1–10
Ham heated	5	−	−	<10
Pepperoni sausage	8	−	+	1–10
Salami sausage	9	−	+	1–10
Pepperoni sausage	22	+	NT	$10^{1.5}$
Salami sausage	22	+	NT	$10^{1.3}$
Pepperoni sausage	104	−	−	<10
Salami sausage	104	−	−	<10

[a] +, Virus recovery; −, no virus recovery.
[b] PFU, Plaque-forming units.
[c] NT, Not tested.

mycin in 1 ml) and placed in a Vortex mixer for 3 to 5 min. The suspension was centrifuged at 800 × *g* for 15 min. A part of the supernatant was diluted 10-fold. Then 0.5 ml of each dilution was inoculated into four PK-15 cell cultures that had been grown on cover slips in Leighton tubes. The inoculated PK-15 cell cultures were examined at 24, 48, and 72 h after inoculation. Cells on the cover slips were fixed and stained with HC conjugate and examined by the fluorescent antibody cell culture technique. Plaques formed by infectious virus on positive cultures were counted. In samples in which reactions were negative, the original 20% suspensions were inoculated into primary swine kidney monolayers, subcultured to PK-15 after 3 days of incubation, and then examined by the fluorescent antibody cell culture technique.

Animal tests. (i) ASF. A 1-g sample of each of the products (ham and salami and pepperoni sausages) was ground up in cell culture media, and 5 ml of a 1:10 dilution was inoculated i.m. into each of two pigs. The temperature of the inoculated pigs was taken daily, and pigs were examined for signs of illness for 34 days. Blood samples were collected from these six pigs at 34 DPI, at which time they and two control animals were inoculated intravenously with approximately 500 50% infectious doses of ASFV. At 60 days after processing, the same procedure was followed, and six pigs were again inoculated with the same products. At 48 h, blood was taken from these six pigs and subpassaged into six additional pigs. Temperatures of these animals were recorded daily, and, 26 days after passage and subpassage, the immunity of the pigs was challenged by i.m. inoculation of 5 ml of ASFV.

(ii) HC. Each of two swine was inoculated i.m. with 1 g of the test product prepared in a 5-ml suspension and sampled at days 1 to 15.

RESULTS

ASF. Virus titer of the meat from the swine infected with ASF ranged from $10^{3.25}$ to $10^{3.75}$ Had_{50}/g in both the whole and ground meat. The salami and pepperoni meat with the additives and starter before being stuffed into the casings contained $10^{2.0}$ through $10^{3.25}$ Had_{50}/g. The virus titer in the brined ham before heating ranged from 10^{2} to $10^{3.75}$ Had_{50}/g, but no virus was recovered from the heated hams. Virus was recovered from the pepperoni sausage after smoking; however, virus was recovered from only one of three samples in the salami sausage after smoking.

Virus was not detected in samples of salami and pepperoni sausages tested approximately 1 month after smoking (Table 1).

Pigs inoculated with the samples at 30 and 60 days after processing did not become infected, and their serum contained no antibody to the virus. All inoculated pigs were susceptible to ASF when challenged.

HC. The virus content of the meat from the swine infected with HCV and slaughtered at 5 DPI ranged from $10^{1.5}$ to $10^{1.87}$ plaque-forming units per g. Minimal HCV infectivity was detected in brined ham, pepperoni and salami meat, and pepperoni and salami sausages. Most of the specimens revealed infectivity only on subculture (Table 3).

The swine inoculated with samples from the carcasses of swine infected with HCV, from the brined ham, and from the ground salami and pepperoni meat with the additives all developed signs and lesions characteristic of HC and died on days 6 and 7. The two swine inoculated with samples from the heated hams remained normal. The swine inoculated with the samples from the pepperoni sausage 15 days after smoking (22 days after slaughter) became infected with HCV and died by day 6. However, both swine inoculated with samples from the salami sausage 14 days after smoking (21 days after slaughter) had temperatures up to 41.6°C, but did not show any other signs of infection. Upon challenge of these two swine by virulent HCV at 14 DPI, one became infected and died; the other had an increased temperature but remained normal

(Table 2). Both control pigs became infected and died upon exposure to a similar inoculation of HCV. Later samples of salami and pepperoni sausages were negative when tested in swine.

DISCUSSION

Cottral et al. (5) found that chemical changes that take place during the ripening of meat inactivate FMDV in muscle tissue, but do not appreciably affect the viruses in lymph nodes, large blood clots, or bone marrow. They concluded that meat derived from animals infected with FMDV was not rendered free of the virus by the usual commercial procedures of ripening, boning, salting, and storage.

Similarly, in Argentina, studies on the survival of FMDV in cured and uncured beef prepared from vaccinated and from unvaccinated cattle indicated a possible risk of virus survival (1).

Graves and McKercher (6) have shown that SVDV can survive in dried salami and pepperoni sausages for at least 400 days and in processed intestinal casings for at least 780 days. However, when partly cooked hams were heated to 69°C, neither SVDV nor FMDV could be recovered.

FMDV could not be recovered from the processed products, although the possibility remains that there could be residual virus in bone marrow or lymph nodes. However, residual FMDV remained in processed intestinal casings for as long as 250 days.

The treatment of the processed casings from swine infected with SVD or FMD by 0.5% lactic or citric acid was effective against FMD. Lactic acid was the more effective with SVDV; however, the virus resisted exposure to citric acid for 24 h.

In the present study, ASFV was recovered from brined ham and processed and smoked salami and pepperoni sausages. No virus infectivity was detected beyond 30 days in fully processed products either by tissue culture or by animal inoculation (Table 1). Thus, these pork products produced in countries where ASF is present would not likely be sources of infection, as the virus was not isolated beyond the processing period. Products such as salami and pepperoni sausages are processed in the drying room for at least 25 and 16 days, respectively.

The HCV in infected meat withstood processing, but samples taken shortly after the curing period (beyond 15 days) were negative, as indicated by animal and tissue culture isolation (Tables 2 and 3).

The work described here has dealt with only a limited number of products that could prove potential hazards. Also, the amount of these viruses in the products would be very small unless the products were prepared from meat from a herd undergoing active infection or possibly an inapparent infection. The cumulative effect of the interactions of food components, temperature, time of exposure, and type may or may not aid in virus survival. No attempt has been made at this time to study anything but primary contamination, but such contamination is in itself a serious threat to the foundation stock of our national multibillion dollar livestock industry and its relationship to international commerce.

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