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Survival of foot-and-mouth disease, African swine fever, and hog cholera viruses in Spanish serrano cured hams and Iberian cured hams, shoulders and loins

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The survival of foot-and-mouth disease virus (FMDV), African swine fever virus (ASFV), and hog cholera virus (HCV) was studied in typical Spanish dry cured meat products (Serrano hams and Iberian hams, loins, and shoulders). For each disease, 31 to 35 Iberian black and 31 or 32 white pigs were infected and slaughtered in Spain at the estimated peak of viremia. Cuts from the carcasses were frozen, shipped to the US and used to prepare the meat products tested. Samples taken at the time of slaughter and at intervals during the processing were assayed for virus survival by in vitro *and* in vivo *techniques. The Iberian hams were free of viable FMDV by day 168, free of viable ASFV by day 140, and free of viable HCV by day 252. The Iberian shoulder hams were free of viable FMDV by day 112, ASFV by day 140, and HCV by day 140. The Iberian loins were free of viable FMDV by day 42, ASFV by day 112, and HCV by day 126. The white Serrano hams were free of viable FMDV by day 182, ASFV by day 140, and HCV by day 140. This work tested industrial procedures to assure that importation and commercialization of these dry cured meat products will not pose a risk to US livestock.*

Introduction

Iberian style curing of pork products is a controlled salting and long-term drying process used in Spain to produce Iberian cured hams, shoulder hams, and loins, and the white Serrano ham. Importation of these products to the US is currently prohibited because of limited knowledge of the effect of the dry curing

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process on inactivation of livestock disease viruses which are not present in the US. The USDA considers Spain to be affected with African swine fever (ASF), foot-and-mouth disease (FMD), and hog cholera (HC) (classical swine fever).

In 1989, the Ministry of Agriculture, Fisheries and Nutrition of Spain (MAPA) and the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) agreed to test the Iberian dry curing process for the inactivation of selected porcine disease viruses. In this report we shall report the effect of the Iberian dry curing process on the inactivation of FMD virus, ASF virus, and HC virus.

Materials and Methods

Animals

The products tested were Iberian ham, shoulder ham, and loin produced from the Spanish black pig and the Serrano ham produced from the domestic white pig. Animal inoculation and slaughter were done in Spain because the live black pigs could not be imported into the United States. The black pigs weighed between 133 and 162 kg, and the white pigs weighed between 92 and 108 kg. Pigs were obtained from FMD, ASF, and HC seronegative commercial Spanish herds that produce pigs for production of the Iberian products and Serrano hams. Animal inoculation was done in the high security animal facilities of the Sobrino Laboratory, American Cyanamid Company, Olot, Spain. The animal facility was decontaminated between each of the diseases studied.

Viral inocula

Foot-and-mouth disease virus was serotype C, Dutch origin, lot 10/89, passed five times in MVPK cell line. Its infectivity was titrated in triplicate using MVPK cells; the titer was determined to be $10^{8.9}$ $TCID_{50}$ ml^{-1}. Inoculum used in this study consisted of 1 ml of 1:10 dilution of the stock virus injected intravenously.

The African swine fever virus used was the fifth buffy coat passage of INIA E-70, lot D-89. The inoculum consisted of 1 ml of undiluted stock virus ($10^{6.5}$ $TCID_{50}$ ml^{-1}) injected intramuscularly.

The hog cholera virus used was the Ames strain in defibrinated blood, lot 86–07. The infectivity of the HCV was tritrated in triplicate by inoculation onto PK-15 cell coverslip cultures. The inoculated cultures were incubated for 5 days, fixed in acetone, stained with anti-HCV conjugate, and examined for fluorescing plaques. The inoculum titer was $10^{5.3}$ $TCID_{50}$ ml^{-1}. One ml of a 1:100 dilution of this stock virus was injected intravenously.

Animal studies

Four non-infected pigs were processed for control products. Each disease was produced separately, and the number of swine inoculated in each disease study were FMD, 31 black pigs and 31 white pigs; ASF, 35 black pigs and 32 white pigs; HC, 32 black pigs and 32 white pigs. All inoculations were done by APHIS personnel. In order to obtain meat with high viral titers, the timing of slaughter was based on knowledge of the disease process using number of days post-inoculation (DPI) and body temperature. In this study, the FMD-infected pigs were slaughtered at 2 DPI, the ASF-infected pigs at 5 DPI, the HC-infected white pigs at 5 DPI, and the HC-infected black pigs at 4 DPI.

Commercial methods, equipment, and the personnel used normally in slaughterhouses producing these types of dry cured meats were used. The slaughter, breaking of the carcass, and trimming of the cuts were done under the supervision of meat technologists from the industry. The pigs were stunned by electric shock and then terminally bled from the anterior vena cava. At the time of slaughter, a heparinized blood sample was collected for infectivity assay. Lesions were photographed. The carcasses were dehaired by placing in a 60°C (140°F) scalding vat for 5 min followed by scraping. The carcasses were eviscerated and halved. Muscle, fat, bone marrow, and lymph node were collected from each animal. All the blood and tissue samples were placed in dry ice and shipped to the Foreign Animal Disease Diagnostic Laboratory (FADDL), Plum Island Animal Disease Center (PIADC), for assay. The carcasses were hung in a 4°C (39°F) cooler overnight. Paired cuts to be processed were labeled by a color code for the disease, pig number, and left or right part of the animal. The cuts were hung in a –40°C (–40°F)

freezer for 24 h, then placed into individual plastic bags and packed in stainless steel containers that were held up to 6 months at –40°C until shipped to PIADC. For shipment, the containers were placed in a low temperature land–sea container and shipped by boat. The meat was held at –39°C (–38°F) during shipment. At PIADC, the containers were moved into the containment laboratory. The frozen cuts for production of the Iberian hams, shoulders, and loins and the Serrano hams were hung in separate environmental chambers for controlled thawing. The processing of the products was under the supervision of designated Spanish meat technologists who followed previously approved protocols. Day 0 of processing was the time the product was thawed.

Four environmental chambers and computerized temperature and humidity controls were provided by MAPA to process the products. A record of processing parameters for each product was kept by the computer and on chart paper. The processing was monitored by the Spanish technologists through daily telecopies of chamber parameters and by regular visits to observe the progress of the processing. The minimum commercial curing period, which begins with salting, for each of these products is 365 days for Iberian cured hams, 270 days for Iberian cured shoulder hams, 90 days for Iberian cured loins, and 190 days for Serrano hams.

Collection and in vitro *assay of tissue samples during processing*

Tissue samples for infectivity assay were aseptically collected from three of each product at predetermined intervals. The products for assay were randomly selected, and cuts from the left and right sides were sampled at alternate times. Muscle, fat, popliteal lymph node and bone marrow samples were collected from hams. Muscle, fat and bone marrow samples were collected from shoulders. Loin samples were muscle and usually a small amount of fat. Muscle samples were taken from the center of thick areas so as to obtain the most moist, least cured sample. Fat samples were taken from beneath the skin. Samples were held for up to 1 week at 4°C until processed. One gram of each sample was ground using a Virtis grinder, mortar and pestle with sterile alundum, or a Tenbrouck grinder and 9 g of Eagle's minimum essential medium containing gentamicin (30 mg l^{-1}) and amphotericin B (2·5 mg l^{-1}). The suspension was centrifuged at 600 *g* for 20 min, and the supernatant collected and used as the inoculum. A 0·1 ml sample of the supernatant was inoculated onto cell cultures, and the remainder of the supernatant and the unground sample were frozen and stored at –70°C. Infectivity assay for each FMD virus sample was performed using lamb kidney cells in 6 well plates overlaid with methyl-cellulose for the plaque assay and in two 25 cm^2 flasks. If cytopathic effect (CPE) was not observed in 4–5 days, material from the flasks was passaged an additional two times in flasks. Each sample for ASFV was cultured on porcine buffy coat cultures in 24 well plates. If hemadsorption was not observed in 2–5 days, a total of 2 blind passages were made. Each sample for HCV was inoculated into chambered slides and two T-25 flasks of PK-15 cells; if no immuno-fluorescence for HC was observed in the first passage, a second passage was made in flasks and incubated 48 h. The third passage on chambered slides was read by immunofluorescence after a 1 day incubation.

In vivo *assay*

When three consecutive samples of a product were negative for a disease by *in vivo* assay, the frozen supernatants of the last three consecutive negative samples (hams for example, muscle, fat, bone marrow and lymph node) were thawed and pooled for *in vivo* assay. The pool was made using all of the earliest sample, two-thirds of the next sample, and one-third of the latest sample. The reason for this pooling of samples was that if the test failed, there would be sufficient material left to make a pool of second, third and fourth negative samples. The pool was clarified by centrifugation at 600 *g* for 10 min. The supernatant was injected intradermally for FMD virus detection or intramuscularly for ASFV or HCV detection into two 30 kg pigs. If the pigs became sick, virus isolation was attempted. If the pigs remained clinically normal for 4 weeks, they were bled and the serum tested for antibody to the disease of concern.

Results

The FMDV titers expressed as the inverse of the log plaque forming units (PFU) ml^{-1} in blood, muscle, fat, bone

Table 1. Foot-and-mouth disease viral titers in tissues of Iberian black pigs at slaughter.

Pig no.	Blood	Lymph node	Bone marrow	Fat	Muscle
AB 1	5·0	4·1	3·2	1·0	0·0
AB 2	4·2	4·2	2·6	1·0	0·0
AB 3	4·8	4·2	2·8	0·0	0·0
AB 4	5·2	4·2	3·7	1·0	0·0
AB 5	5·3	6·0	3·3	0·0	0·0
AB 6	4·1	4·4	2·7	1·3	0·0
AB 7	4·3	3·6	2·3	1·9	0·0
AB 8	5·0	4·1	3·6	1·5	0·0
AB 9	5·2	5·3	5·0	2·3	0·0
AB 10	5·1	3·7	2·2	0·0	0·0
AB 11	3·0	4·4	2·9	0·0	0·0
AB 12	5·2	5·6	4·2	1·5	0·0
AB 13	4·9	5·1	5·1	2·0	0·0
AB 14	4·5	3·0	3·0	0·0	1·0
AB 15	4·8	4·1	3·3	0·0	0·0
AB 16	4·7	4·9	2·7	0·0	0·0
AB 17	4·5	3·8	3·8	0·0	1·0
AB 18	4·9	4·7	2·0	1·3	0·0
AB 19	4·8	2·8	2·9	0·0	0·0
AB 20	0·0	0·0	0·0	0·0	0·0
AB 21	4·8	3·6	2·0	1·7	0·0
AB 22	1·7	3·4	0·0	0·0	0·0
AB 23	4·1	2·6	2·3	0·0	0·0
AB 24	4·1	4·6	2·3	1·3	0·0
AB 25	4·5	4·1	3·0	3·7	0·0
AB 26	5·1	5·2	3·4	0·0	0·0
AB 27	5·0	4·7	4·6	1·8	0·0
AB 28	5·1	4·1	3·4	0·0	0·0
AB 29	3·4	4·0	0·0	1·3	0·0
AB 30	3·4	4·4	2·0	0·0	0·0
AB 31	3·4	4·6	0·0	0·0	0·0
Average titer	4·3	4·1	2·7	0·8	0·1

FMDV titers are expressed as the inverse $\log_{10}$ plaque forming units (PFU) ml^{-1} or g^{-1}.

marrow and lymph node at the time of slaughter for each pig are presented in Tables 1 and 2. The mean FMD virus infectivity titers in each tissue of the black and white pigs were highest in the blood (3·6) and lymph nodes (3·4), moderate in the bone marrow (1·9), very low in the fat (0·5), and variably detected in the muscle (0·03). The mean titer from the blood of the black pigs (4·3) was greater than that in the white pigs (2·8). The FMDV persistence (Table 3) in the Serrano ham lymph node and fat correlated with collection of hemorrhagic samples. FMD virus was not detected in muscle after 14 days of processing but persisted up to 84 days of processing in bone marrow. The negative *in vitro* results were confirmed *in vivo* using a pool of the first three consecutive *in vitro* negative samples.

The ASFV titers in blood, muscle, fat, bone marrow and lymph node at the time of slaughter for each pig are pre-

Table 2. Foot-and-mouth disease viral titers in tissues of Spanish white pigs at slaughter.

Pig no.	Blood	Lymph node	Bone marrow	Fat	Muscle
AW 1	2·6	0·0	0·0	1·0	0·0
AW 2	4·3	3·7	2·0	0·0	0·0
AW 3	3·2	3·3	0·0	0·0	0·0
AW 4	4·2	4·4	1·3	0·0	0·0
AW 5	3·5	4·7	1·0	0·0	0·0
AW 6	4·8	4·2	3·7	1·3	0·0
AW 7	4·9	4·2	4·2	2·0	0·0
AW 8	2·0	4·3	1·0	0·0	0·0
AW 9	0·0	0·0	0·0	0·0	0·0
AW 10	2·3	3·5	1·0	0·0	0·0
AW 11	0·0	0·0	0·0	0·0	0·0
AW 12	1·8	3·5	1·0	0·0	0·0
AW 13	2·9	4·6	0·0	0·0	0·0
AW 14	3·1	2·3	1·0	0·0	0·0
AW 15	4·7	4·4	3·6	0·0	0·0
AW 16	4·7	3·3	2·5	1·0	0·0
AW 17	2·2	0·0	0·0	0·0	0·0
AW 18	3·3	3·0	1·5	0·0	0·0
AW 19	2·7	4·3	0·0	0·0	0·0
AW 20	2·9	4·3	1·3	0·0	0·0
AW 21	2·8	3·4	1·3	0·0	0·0
AW 22	4·2	4·0	2·3	0·0	0·0
AW 23	4·1	2·6	2·3	0·0	0·0
AW 24	3·5	3·1	1·3	1·3	0·0
AW 25	1·0	3·5	0·0	0·0	0·0
AW 26	2·0	0·0	0·0	0·0	0·0
AW 27	0·0	0·0	0·0	0·0	0·0
AW 28	2·0	0·0	0·0	0·0	0·0
AW 29	2·1	3·0	0·0	0·0	0·0
AW 30	1·9	0·0	0·0	0·0	0·0
AW 31	2·0	0·0	0·0	0·0	0·0
Average titer	2·8	2·6	1·0	0·2	0·0

FMDV titers are expressed as the inverse $\log_{10}$ plaque forming units (PFU) ml^{-1} or g^{-1}.

sented in Tables 4 and 5. The mean ASFV titers in each tissue of the black and white pigs were highest in the bone marrow (9·5), slightly lower in the lymph node (8·5), somewhat lower in the blood (7·8), and substantially lower in the fat (5·4) and muscle (6·6). The white and black pigs responded similarly to ASFV. The persistence of ASFV (Table 6) was rather uniform, 84 to 112 days of processing, in the fat, muscle and bone marrow of the four products. The virus persistence in lymph node samples from the Iberian hams was longer (112) than in lymph node samples from the Serrano hams (56 days). All the *in vitro* results were confirmed by *in vivo* testing.

The HCV titers in blood, muscle, fat, bone marrow and lymph node at the time of slaughter for each pig are presented in Tables 7 and 8. The mean HCV titers in each tissue of the black and white pigs were highest in the bone marrow (5·2), somewhat lower in the

Table 3. Inactivation of foot-and-mouth disease virus during processing of Iberian and Serrano products.

Product	Muscle	Fat	Bone marrow	Lymph node	*In vivo* test
Iberian ham	0	0[a]	56	112	168 – 196 – 224
Iberian shoulder	0	0	84		112 – 140 – 168
Iberian loin	0				42 – 56 – 70
Serrano ham	0	140[a]	84	168[b]	182 – 196 –210

The numbers under muscle, fat, bone marrow and lymph node represent the day of processing that the last sample was positive *in vitro* for FMDV. The numbers under *in vivo* testing represent the day of processing for the samples in the pool inoculated.
[a]The 140 day sample was hemorrhagic and FMDV was isolated.
[b]The 168 day samples from two hams were hemorrhagic, and FMDV was isolated.

Table 4. African swine fever viral titers in tissues of Iberian black pigs at slaughter.

Pig no.	Blood	Lymph node	Bone marrow	Fat	Muscle
AB 1	9·9	7·9	8·4	7·2	7·7
AB 3	9·9	8·4	9·2	5·4	6·9
AB 5	9·9	8·4	9·4	5·2	7·7
AB 6	9·9	7·9	10·9	3·9	7·4
AB 7	9·9	8·7	7·9	4·9	6·9
AB 8	9·9	8·4	10·2	3·7	6·7
AB 9	9·9	8·4	8·9	5·9	7·4
AB 10	9·9	8·7	9·7	5·4	6·4
AB 11	9·9	9·2	10·4	5·7	6·9
AB 12	7·2	7·9	9·2	4·7	6·2
AB 13	8·4	8·4	9·2	5·2	6·2
AB 15	6·7	8·2	8·2	4·4	6·7
AB 16	9·9	8·4	8·9	4·9	6·4
AB 17	8·9	9·4	9·2	3·9	6·7
AB 18	6·9	8·2	10·2	4·4	6·2
AB 19	9·4	8·2	8·7	5·4	6·2
AB 20	7·9	8·2	8·9	4·7	6·4
AB 21	7·9	9·4	8·7	4·9	6·7
AB 22	8·2	7·9	8·9	4·9	6·4
AB 23	7·4	9·2	8·9	4·2	6·4
AB 24	7·2	9·4	9·4	4·9	6·2
AB 25	6·2	6·4	8·4	4·4	5·2
AB 26	6·9	9·2	9·2	4·7	6·4
AB 27	9·7	9·7	8·9	4·7	5·9
AB 28	7·4	8·7	9·4	5·2	6·4
AB 29	7·7	7·9	9·4	5·2	6·7
AB 30	6·5	8·9	9·7	4·2	5·7
AB 31	6·2	7·9	10·9	4·4	5·9
AB 32	7·2	7·9	8·9	4·7	5·9
AB 33	6·5	10·2	9·2	4·7	6·2
AB 34	9·9	9·7	8·7	5·2	5·9
AB 35	5·9	8·9	9·2	5·2	5·9
Average titer	8·3	8·6	9·2	4·9	6·5

ASFV titers are expressed as the inverse $\log_{10}$ per g or ml.

Table 5. African swine fever viral titers in tissues of Spanish white pigs at slaughter.

Pig no.	Blood	Lymph node	Bone marrow	Fat	Muscle
AW 1	6·2	6·9	10·2	6·8	6·4
AW 2	6·9	7·6	9·2	6·5	6·4
AW 3	7·4	7·2	9·4	5·8	6·7
AW 4	8·4	8·4	8·4	4·9	5·9
AW 5	7·7	8·2	8·7	6·8	6·4
AW 6	8·4	8·2	9·7	5·7	6·4
AW 7	7·9	9·4	9·2	5·7	6·7
AW 8	8·2	8·4	10·4	5·9	5·9
AW 9	7·4	8·9	10·4	6·7	7·2
AW 10	8·4	6·9	7·7	6·4	7·2
AW 11	6·7	7·9	10·7	6·4	7·2
AW 12	8·2	8·7	9·9	7·4	7·2
AW 13	7·7	8·2	9·9	6·9	6·7
AW 14	6·9	8·4	10·2	6·4	7·2
AW 15	8·4	8·7	10·9	7·2	7·4
AW 16	7·7	7·7	8·9	7·4	7·7
AW 17	6·9	7·7	10·4	5·6	5·9
AW 18	8·4	7·4	9·7	5·5	6·3
AW 19	8·7	7·7	10·4	5·3	6·3
AW 20	8·4	7·2	9·2	5·2	6·3
AW 21	6·2	10·9	10·7	5·5	7·3
AW 22	7·1	10·9	10·9	4·4	6·5
AW 23	8·4	7·7	9·2	5·5	7·0
AW 24	6·7	10·9	9·9	5·8	6·3
AW 25	7·2	8·4	10·4	4·9	6·8
AW 26	5·9	10·9	10·7	5·5	6·9
AW 27	6·7	10·9	9·2	5·3	6·2
AW 28	7·2	7·4	7·9	4·4	6·2
AW 29	8·2	10·9	8·9	5·7	7·0
AW 30	8·4	6·9	9·7	5·3	6·9
AW 31	7·9	9·7	10·7	4·9	5·9
AW 32	7·2	6·2	8·9	5·3	6·9
Average titer	7·6	8·5	9·7	5·8	6·7

ASFV titers are expressed as the inverse $\log_{10}$ per g or ml.

Table 6. Inactivation of African swine fever virus during processing of Iberian and Serrano products.

Product	Muscle	Fat	Bone marrow	Lymph node	*In vivo* test
Iberian ham	112	112	112	112	140 – 168 – 196
Iberian shoulder	112	84	84		140 – 168 – 196
Iberian loin	98				112 – 126 – 140
Serrano ham	112	112	112	56	140 – 154 – 168

The numbers under muscle, fat, bone marrow and lymph node represent the day of processing that the last sample was positive *in vitro* for FMDV. The numbers under *in vivo* testing represent the day of processing for the samples in the pool inoculated.

Table 7. Hog cholera viral titers in tissues of Iberian black pigs at slaughter.

Pig no.	Blood	Lymph node	Bone marrow	Fat	Muscle
HB 01	4·4	+	5·5	+	+
HB 02	4·7	4·0	5·5	+	+
HB 03	5·3	+	6·2	+	+
HB 04	5·3	4·3	6·2	+	+
HB 05	4·5	1·0	4·6	+	+
HB 06	4·5	4·0	6·1	+	+
HB 07	2·4	3·5	4·8	+	+
HB 08	6·0	4·3	6·0	+	+
HB 09	5·2	3·6	6·1	+	+
HB 10	4·4	3·3	5·8	+	+
HB 11	4·1	3·5	5·6	+	+
HB 12	5·6	4·8	6·1	+	+
HB 13	4·5	3·4	5·4	+	+
HB 14	4·4	3·8	6·2	+	+
HB 15	4·0	3·4	6·0	+	+
HB 16	4·6	4·1	6·1	+	+
HB 17	1·0	4·6	4·5	+	+
HB 18	5·4	4·5	6·5	+	+
HB 19	5·0	4·4	6·8	+	+
HB 20	3·9	5·0	6·3	+	+
HB 21	5·0	5·6	6·1	+	+
HB 22	3·3	4·2	5·0	+	NEG.
HB 23	1·0	3·7	3·8	NEG.	+
HB 24	1·0	3·5	4·5	NEG.	NEG.
HB 25	2·4	3·0	5·0	NEG.	NEG.
HB 26	2·3	4·3	5·5	+	+
HB 27	3·2	4·7	5·5	+	+
HB 28	4·4	4·3	6·6	+	+
HB 29	2·1	4·2	4·5	+	+
HB 30	1·0	4·5	4·7	NEG.	NEG.
HB 31	1·0	3·5	3·7	NEG.	+
HB 32	3·4	4·0	6·7	+	+
Average titer	3·7	3·8	5·6	0·8	0·9

HCV titers are expressed as the inverse of the $\log_{10}$ PFU g^{-1} or ml^{-1}, or as positive (+) if only the lowest dilution was positive after blind passage. Positives were given the value of 1 for calculation of average titer.

lymph nodes (3·8) and blood (3·8), and quite low in the fat (0·9) and muscle (1·0). The white and black pigs responded similarly to HC virus. Hog cholera virus was detected by *in vitro* testing up to 168 days of curing in the Iberian ham muscle and lymph node (Table 8). However, *in vivo* testing of the Iberian hams was positive for HC virus using a pool of supernatant from samples collected on days 196, 224, and 252 of processing. A second *in vivo* test of the Iberian hams using a pool of supernatant from samples collected on days 252, 280 and 343 of processing was negative for HC virus. In contrast, the Serrano ham *in vivo* test was negative for HCV using a pool of supernatant from samples collected on days 140, 154 and 160 of processing.

Table 8. Hog cholera viral titers in tissues of Spanish white pigs at slaughter.

Pig no.	Blood	Lymph node	Bone marrow	Fat	Muscle
HW 01	4·8	5·0	3·7	+	+
HW 02	4·7	5·0	6·0	+	+
HW 03	4·2	4·4	5·5	+	+
HW 04	7·4	4·8	5·5	3·8	3·4
HW 05	5·3	5·2	5·6	3·7	3·5
HW 06	3·8	+	5·3	NEG.	NEG.
HW 07	4·1	+	5·0	+	+
HW 08	3·9	+	5·5	+	+
HW 09	7·3	5·1	6·2	3·9	+
HW 10	4·2	4·3	4·7	3·4	+
HW 11	3·4	4·4	4·5	+	+
HW 12	5·3	4·2	4·0	NEG.	+
HW 13	3·7	4·0	3·7	NEG.	+
HW 14	3·3	4·3	5·0	+	+
HW 15	5·5	+	4·2	NEG.	+
HW 16	3·4	3·6	4·5	NEG.	+
HW 17	5·7	4·2	5·7	NEG.	+
HW 18	3·2	4·4	4·8	+	+
HW 19	4·3	5·2	5·7	+	+
HW 20	4·6	4·3	5·7	+	+
HW 21	4·3	4·6	5·2	+	+
HW 22	4·9	5·5	5·8	+	+
HW 23	+	4·6	4·6	+	+
HW 24	3·2	5·0	5·2	NEG.	+
HW 25	3·4	4·5	5·5	+	+
HW 26	4·5	5·0	6·0	+	+
HW 27	3·0	3·7	3·3	+	+
HW 28	+	4·2	4·6	+	+
HW 29	+	5·1	+	+	+
HW 30	+	3·5	4·4	NEG.	+
HW 31	3·3	4·0	4·9	+	+
HW 32	3·3	4·8	5·5	+	+
Average titer	3·9	4·1	4·9	1·1	1·1

HCV titers are expressed as the inverse of the $\log_{10}$ PFU g^{-1} or ml^{-1} or as (+) positive if only the lowest dilution was positive after blind passage.

Discussion

The purpose of this study was to determine if the Iberian and Serrano dry curing processes would inactivate animal viruses of concern to the USDA. FMD, ASF and HC viruses do not infect man, but historically these viruses have been spread when wastes of food products produced from infected animals were fed to pigs. The use of meat from animals inoculated with the viruses of concern and slaughtered at the expected peak of infection, for testing virus inactivation represented the 'worst case scenario'. The meat from these animals contained the greatest amount of virus that could occur in the product. In reality, animals at this stage of infection should be rejected on ante-mortem inspection and thus not enter the food chain.

Table 9. Inactivation of hog cholera virus during processing of Iberian and Serrano products.

Product	Muscle	Fat	Bone marrow	Lymph node	*In vivo* test
Iberian ham	168	84	84	168	252 – 280 – 343[a]
Iberian shoulder	112	84	112		140 – 168 – 196
Iberian loin	112				126 – 140 – 154
Serrano ham	112	84	84	84	140 – 154 – 168

The numbers under muscle, fat, bone marrow, and lymph node represent the day of processing that the last sample was positive *in vitro* for FMDV. The numbers under *in vivo* testing represent the day of processing for the samples in the pool inoculated.

[a]The *in vivo* test of a pool prepared from days 196, 224, and 252 of processing was positive for hog cholera virus.

Inactivation times for FMDV, ASFV, and HCV in hams produced by the 'Prosciutto di Parma' process, which is also a salting and drying process, as determined by the Italian and US workers, were respectively: FMDV-170 days, ASFV-300 days, HCV-189 days, and FMDV-108 days, ASFV 399 days and HCV 313 days. In other salted/dried products, FMDV survived for 190 days in bacon, 183 days in ham fat, and 89 days in ham bone marrow (Cottral et al. 1960, Savi et al. 1962, Dhennin et al. 1980) and HCV for 70 days in ham bone marrow and 90 days in ham muscle and fat (Savi et al. 1964). This study ot persistence of FMDV, ASFV, and HCV in Serrano and Iberian dry cured products corroborates, in general, the survival times of viruses in meat products and provides additional information on the survival of these viruses in different tissues. While the muscle quickly becomes negative for FMDV (14 days of processing), probably due to the accumulation of lactic acid (Cottral et al. 1960), the virus persisted for a longer time in lymph nodes, bone marrow, and fat of the products in this study.

In the HCV testing, the sensitivity of animal inoculation to detect viable virus was substantiated. The pigs developed HC in the first *in vivo* test of the Iberian ham product. Of the three viruses, HCV persisted the longest in the products tested.

The persistence of ASF in Serrano and Iberian ham (112 days of processing) in this study basically supports Botija's findings of ASFV persisting for 5 to 6 months in muscle and bone marrow (Botija 1982). These results are considerably shorter than the reported 300- and 399-day survival of ASFV in Parma ham (McKercher et al. 1987). This variation in the survival of viruses in different meat products under unique conditions underscores the need to conduct

Table 10. Comparison of range of commercial curing times (days) and processing times (days) of the first sample in the negative *in vivo* test.

Product	Curing time	FMDV	ASFV	HCV
Iberian ham	365–730	168	140	252
Iberian shoulder	240–420	112	140	140
Iberian loin	90–130	42	112	126
Serrano ham	180–365	182	140	140

full studies on the curing process for each product prior to the importation of the product into areas free of diseases that could be present in the area of origin. These studies are required by the USDA prior to granting permission to import a product.

Conclusion

The results of this study demonstrated that FMDV, ASFV, and HCV are inactivated by commercial curing processes. In Table 10, the commercial curing times are compared with the times required for the inactivation of the different viruses in all the cured meat products tested. This information is needed to assure that importation and commercialization of these dry cured meat products will not pose a risk to US livestock and to provide information for regulatory agencies to develop criteria for importation of the products.

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