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Survival of swine vesicular disease virus in Spanish Serrano cured hams and Iberian cured hams, shoulders and loins

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The survival of swine vesicular disease virus (SVDV) was studied in typical Spanish dry cured pork products (Serrano and Iberian hams, loins, and shoulders) to determine if SVDV would be inactivated in products imported from infected pigs and, thus, assure that importation and commercialization of these dry cured meat products would not pose a risk to US livestock. Thirty-two Iberian black and 32 white pigs were infected and slaughtered in Spain at the estimated peak of viremia. Cuts from the carcasses were frozen and shipped to the United States. The meat products tested were prepared in an off-shore high containment laboratory using procedures mimicking commercial processing procedures used in Spain. Samples taken at slaughter and at intervals during processing were assayed for virus survival by in vitro *and* in vivo *techniques.*

At slaughter, virus titers were highest in lymph nodes, moderate in blood and bone marrow, and lowest in fat and muscle. The Iberian loins were free of viable SVDV by day 28. The Iberian shoulder hams were free of viable SVDV by day 112. The Iberian hams were free of viable SVDV by day 560. The white Serrano hams were free of viable SVDV by day 539. The time required for inactivation of the virus in lymph nodes in Serrano hams exceeded the maximum commercial curing time.

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 from the Spanish black pig and the Serrano hams from the white pig. 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

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its effectiveness in inactivation of selected pathogenic porcine viruses. This paper supplements a previous publication (Mebus et al. 1993) in which was reported the inactivation of foot-and-mouth disease, African swine fever, and hog cholera viruses by the Iberian dry curing process. Swine vesicular virus was included in the study because, when the experiment was designed, Spain was not recognized by the United States as being free of SVDV.

Materials and Methods

Viral inoculum, slaughter and processing procedures

The swine vesicular disease virus inoculum was the UKG 72 isolate (Burrows et al. 1974) that had a history of four swine passages and two IB-RS-2 cell passages. The infectivity of the inoculum was titrated in triplicate using IB-RS-2 cell line; the titer was $4{\cdot}5 \times 10^7$ pfu ml^{-1}. APHIS personnel injected 3 ml of 1 : 3 dilution of this stock virus intravenously into susceptible seronegative Spanish black and Spanish white pigs that were then slaughtered 3 days post inoculation (DPI). The timing of the slaughter was based on knowledge of relationship of viral titers in the animal to the disease process and body temperature.

The pigs and meat were processed as previously reported (Mebus et al. 1993). The products tested were Iberian hams, shoulder hams, and loins produced from 32 Spanish black pigs and the Serrano hams produced from 32 Spanish domestic white pigs.

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

Tissue samples were collected, processed, and assayed for infectivity as previously reported (Mebus et al. 1993) using IB-RS-2 cell line. If cytopathic effect (CPE) was not observed in 2–3 days, the culture was frozen at –70°C and thawed at room temperature. Then 0·2 ml was transferred onto a new monolayer and incubated at 37°C. If no CPE was observed in 2–3 days, the process was repeated and the cultures read over a period of 7 days.

In vivo *assay*

Animal inoculations with suspensions for tissues taken at various stages in the curing process were performed as previously reported (Mebus et al. 1993).

Results

The SVDV titers expressed as the inverse of the $\log_{10}$ plaque forming units (pfu ml^{-1} in blood, muscle, fat, bone marrow, and lymph node at the time of slaughter for each pig are presented in Tables 1 and 2. The mean SVD virus ineffectivity titers in each tissue of the black and white pigs were highest in the lymph nodes (6·7 and 5·7, respectively), moderate in the blood and bone marrow of the black pigs and blood of the white pigs, and variable in the fat and muscle of both the black and white pigs. Swine vesicular disease virus was not detected in muscle, fat and bone marrow after 84 days of curing, but persisted for at least 470 days of processing in lymph nodes (Table 3). The negative *in vitro* results were confirmed *in vivo* using a pool of the first three consecutive *in vitro* negative samples. Comparison of commercial curing times (days) and processing times (days) when curing samples first tested negative for SVDV by *in vitro* methods (Table 4) revealed that the time required for inactivation of the virus in lymph nodes in the Serrano hams exceeded the maximum commercial curing time, and the time required for the inactivation of the virus in lymphoid tissue of the Iberian hams approached the maximum commercial curing time.

Discussion

This study was designed to determine if Iberian and Serrano dry curing processes inactivate SVDV. Historically,

Table 1. Swine vesicular disease viral titers in tissues of Iberian black pigs at slaughter.

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

[a] $\log_{10}$ plaque forming units (pfu) ml^{-1} or g^{-1}.

SVDV has been spread by wastes of food products produced from infected animals fed to pigs. The use of meat from animals inoculated with SVDV and slaughtered during the expected peak of acute infection represented the 'worst case scenario' because this meat contained the greatest possible amount of virus. In reality, animals at this stage of infection should be rejected on antemortem inspection and thus not enter the food chain.

In a joint Italian–US project (McKercher et al. 1985), hams produced by the 'Prosciutto di Parma' process, which is also a salting and drying process, were negative on culture for SVDV at 182 and 310 days in the Italian study and at 300 and 360 days in the US contribution to the project. In another

Table 2. Swine vesicular disease viral titers in tissues of Spanish white pigs at slaughter.

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

[a]$\log_{10}$ plaque forming units (pfu) ml^{-1} or g^{-1}.

Table 3. Survival (in days) of swine vesicular disease virus during processing of Iberian and Serrano products.

Product	Muscle	Fat	Bone marrow	Lymph node	*in vivo* test
Iberian loin	14[a]	—	—	—	42 – 56 – 70[b]
Iberian shoulder	14	84	84	—	196 – 224 – 238
Serrano ham	84	84	84	470	539 – 560 – 574
Iberian ham	84	84	84	470	560 – 574 – 589

[a]Number of days sample was found positive for SVDV by *in vitro* methods.
[b]Days when each of three samples were pooled for *in vivo* testing.

Table 4. Comparison of range of commercial curing times (days) and processing times (days) of the first sample in the negative *in vitro* test.

Product	Curing time[a]	Processing time[b]
Iberian ham	365–730	560
Iberian shoulder	240–420	112
Iberian loin	90–130	28
Serrano ham	180–365	539

[a]Range of days that the product is cured during commercial production.
[b]Day of processing that the first sample was negative for SVDV.

study on salted/dried products, SVDV survived for 400 days in dried pepperoni and salami sausages and 780 days in processed intestinal casings (McKercher et al. 1974, 1980). In the present study of the effect of the Spanish dry cure process on the persistence of SVDV, the viral titers in preprocessed and processed tissues more closely approximated the viral titers in the Italian 'Prosciutto di Parma' study; this most likely resulted from the similarity in methods of slaughter. Stunning and bleeding results in lower viral titers in muscle than does Pentothal anesthesia and bleeding. The effect of method of slaughter on viral titers in tissues was discussed in the 'Prosciutto di Parma' paper (McKercher et al. 1985). The primary difference in the 'Prosciutto de Parma' and Spanish dry cure experiments was the difference in the persistence of SVDV in lymph nodes. In the Italian phase of the 'Prosciutto de Parma' study, popliteal lymph nodes cultured at 310 and 395 days were negative for SVDV while, in the Spanish dry cure experiments, SVD persisted for 470 days. This difference may have been due to initial high titers and increased number of pigs sampled in the Spanish dry cure experiment. The average SVDV titer in lymph nodes from three pigs at the time of slaughter in the Italian phase of the 'Prosciutto di Parma' study was $10^{4.7}$ pfu g^{-1}, while the average titers in the lymph nodes of the Spanish white pigs and Iberian black pigs were $10^{5.7}$ and $10^{6.7}$ pfu g^{-1}, respectively. In addition, it appears that lymph nodes were only cultured from 20 pigs in the Italian phase of the experiment while, in the Spanish dry cure experiments, lymph nodes were cultured from 64 pigs.

This variation in the survival of exotic viruses in different tissues and meat products under unique conditions underscores the need to conduct detailed studies on the processing of each product prior to its importation into disease-free areas. These studies are required by the USDA prior to permitting importation of a product.

Conclusion

This study demonstrated that SVDV is inactivated by commercial curing processes, and levels of virus inactivation are directly related to curing time. However, the time required for inactivation of SVDV in lymphoid tissue in the Serrano ham exceeded the maximum commercial curing time, and SVDV survival in lymph nodes of Iberian hams approached the maximum commercial curing time. This viral resistance must be considered when developing importation regulations for products to assure they do not pose a risk to US livestock.

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