FATS AND PROTEINS RESEARCH FOUNDATION, INC.

Director's Digest

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STABILITY OF THE PSEUDORABIES VIRUS (PRV) IN MEAT AND BONE MEAL (MBM) AND INTERMEDICATE RENDERING PRODUCTS

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For several years pre-mix sales personnel and uninformed veterinarians have suggested to swine producers that animal proteins could be a carrier of the pseudorabies virus. Members of the Pseudorabies Committee of the U.S.A.H.A. and other virologists have repeatedly stated that the pseudorabies virus could not survive rendering temperatures. To substantiate their belief that animal proteins could not be implicated in the spread of pseudorabies the Central Region of the National Renderers Association sponsored a research project with Dr. Eugene C. Pirtle at Iowa State University that studies the stability of the pseudorabies virus (PRV) in meat and bone meal (MBM) and intermediate rendering products. (Pirtle, 1990).

Twenty swine weighing approximately 115 lbs. (52.3 kg) each were intranasally infected with 2 ml of a virulent strain of PRV which had a titer of 1×10^8 median cell culture infectious doses per milliter.

These animals were rendered as a separate lot and marked with a red dye. Twelve samples were taken at seven collection points. The results of culturing swine tissues are in Table 1. No PRV was isolated from the swine tissues using cell culture methods. Because swine tissue preparations from collection points 1 and 2 were toxic for cell cultures, supernatant fluids from collection points 1, 2 and 3 were thawed from storage at minus 80°C and three inoculum pools were made from samples 1, 3, 5, 7, 9 and 11 from the three collection points. Three groups of ten Balb C mice were inoculated intraperitonially with 0.5 ml of each of the three inoculum pools and observed for 12 days. All thirty mice remained healthy during the observation period.

There are several reasons why PRV was not isolated from PRV - infected swine tissues in this worst-case scenario. In adult swine pseudorabies mainly involves the respiratory and central nervous system tissues, a relatively small fraction of the total body weight.

It is estimated that dilution alone reduced virus-laden tissues at least 10^3 or 10^4.

The pH of samples of the first three collection points were 6.3 for point 1 and 6.31 for point 2 and 6.93 for point 3. Exposure of PRV to the relatively acid environments from points 1 and 2 were not compatible with optimal conditions for PRV survival. It also appears that the initial steps of the rendering process artificially released autolytic products and enzymes responsible for observed toxic effects on cell cultures. These same substances might also have had toxic and inactivating effects on the virus particles of PRV.

To demonstrate the toxic effect of unheated raw material on the PRV virus, Dr. Pirtle contaminated samples from collection point 1, 2

and 3. (Table 2). Tissues from collection points 1 and 2 were both toxic for cell cultures either alone or with PRV added to the tissues. However, there were many intact cells in monolayers at 24 hours in the case of sample 3, but no PRV CPE was observed when there was marked PRV CPE in cultures of the PRV only control. It thus appeared that the effects of toxicity were present at 24 hours even though the effects were not clearly evident. The emerging toxicity was quite likely viricidal to all dilutions of PRV even before toxicity was observed microscopically. The cytopathic effects of PRV were evident in supernates of PRV contaminated tissues from collection point 3 (this sample was heated to 165°F before contaminated). This result was not unexpected since the pH of tissue from this collection point was 6.96 and had earlier been shown not to be toxic for cell cultures. The results of this experiment demonstrate however that PRV would not have survived through collection points 1 and 2 to later be isolated from tissues from collection point 3.

During the various steps leading to the producion of MBM, the material entering the plate contact dryer is subjected to gradually increasing levels of heat ranging from 165°F (73.9°C) to an exiting temperature of 233.6°F (112°C).

Five millimeter aliquots of undiluted PRV (2×10^8 $CCID_{50}$ml) in cell culture fluid were subjected to the above two temperatures in 10-minute increments, ranging from 0 to 60 minutes. The heated virus fluids were inoculated into cell culture monolayers and observed daily for three days for evidence of CPE. The only cultures showing evidence of CPE were the unheated samples (0-time). PRV was thus inactivated at both temperatures during the first 10 minutes of heating.

Enough liquid (pH 7.0 buffered 0.9 M NaCl) was added to MBM to endow MBM with 50% moisture - the moisture equivalent of material entering the plate contact dryer in the actual operation. Five gram

aliquots of MBM were contaminated with PRV virus and PRV-MBM mixtures were heated at 165°F for 0, 10, 20, 40 and 60 minutes. It was concluded that 165°F (73.9°C) was effective in completely inactivating PRV in as little as 10 minutes when MBM contained 50% moisture when heated.

An experiment was done to determine whether PRV could be recovered from experimentally contaminated MBM after short storage at 77°F (25°C, room temperature). The results of this experiment are presented in Table 3.

It is obvious from this experiment that PRV added to MBM was reduced to undetectable levels within four days, even in the 10^1 dilutions of the virus control after both 4 and 8 days at room temperature. The viability of PRV is adversely affected by drying and it appears that the relatively high input PRV in the 10^1 dilution of the stock virus in this experiment was rapidly dehydrated and thus inactivated.

Twelve different lot numbers of finished MBM produced during a 3 month period were tested for the presence of PRV. A weighed amount of each MBM lot was made into a 10% suspension (w/v) by blending (with minimal warming) followed by centrifugation. Supernatant fluids were inoculated into eight replicate swine kidney cell cultures and observed at daily intervals for evidence of CPE during a four-day period.

No evidence of CPE due to PRV nor toxicity to the cell monolayers was observed.

Dr. Pirtle concluded that from the results obtained in the worst-case scenario in experiment 1 through the monitoring of the final MBM product in experiment 6 that there is little or no possibility that PRV can survive the rigorous processing steps leading to the production of MBM.

REFERENCES

1. Pirtle, Dr. Eugene C., Research Virologist, Iowa State University. "Stability of Pseudorabies Virus (PRV) In Meat and Bone Meal and Intermediate Rendering Products" 1990.

Table 1. Results of culturing swine tissue samples in cell cultures (the worst-case scenario).

Collection Point	Observation in: Cell culture	Mice*
1. Metal detection belt	Inocula toxic for cell cultures	0/10
2. Hasher	Inocula toxic for cell cultures	0/10
3. Press	No cytopathic effects	0/10
4. Scraper tank	No cytopathic effects	
5. Dryer	No cytopathic effects	
6. Fat	No cytopathic effects	
7. Stick-water	No cytopathic effects	

*Number of mice dead over number of mice inoculated.

Table 2. Results in cell culture of culturing experimentally PRV-contaminated tissues, virus dilutions alone, and control tissues alone.

Sample	Observation in PRV dilution: 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
1. Collection point 1 Tissue + PRV	TFC[a]	TFC	TFC	TFC	TFC	TFC
2. Collection point 1 Tissue alone	TFC	TFC	TFC	TFC	TFC	TFC
3. Collection point 2 Tissue + PRV	NCPE[b]	NCPE	NCPE	NCPE	NCPE	NCPE
4. Collection point 2 Tissue alone	TFC	TFC	TFC	TFC	TFC	TFC
5. Collection point 3 Tissue + PRV	4/4[c]	4/4	4/4	4/4	4/4	0/4
6. Collection point 3 Tissue alone	NCPE	NCPE	NCPE	NCPE	NCPE	NCPE
7. PRV alone	4/4	4/4	4/4	4/4	4/4	4/4

[a]Toxic for cell cultures, 24 and 48 hr readings.
[b]No cytopathic effects, 24 hr (3.) and 48 hr (6.) readings.
[c]Number of cell cultures with CPE over number of cell cultures inoculated.

Table 3. Survival of pseudorabies (PRV) in meat and bone meal (MBM) after storage at 77°F (25°C, room temperature).

Sample	Virus dilution	Cytopathic effect	
		Control	MBM+PRV
Day 0	10^{-1}	4/4*	4/4
	10^{-3}	4/4	4/4
	10^{-6}	0/4	0/4
Day 4	10^{-1}	4/4	0/4
	10^{-3}	0/4	0/4
	10^{-6}	0/4	0/4
Day 8	10^{-1}	4/4	0/4
	10^{-3}	0/4	0/4
	10^{-6}	0/4	0/4

*Number of cultures having CPE over number of cultures inoculated.