ENTEROVIRUS PERSISTENCE IN SAUSAGE AND GROUND BEEF

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ABSTRACT

Persistence of coxsackievirus type A9 suspended in ground beef was found not to be sufficiently affected by extensive bacterial growth, during periods of up to 8 days at 23 or 4 C, to afford any notable degree of protection to the consumer. Longer storage times resulted in marked virus loss. After 2 weeks at both temperatures >90% of the input virus was no longer infective. In the preparation of Thuringer sausage, approximately 85% of the input virus was lost during a 24-hr fermentation at 30 C. Subsequent heating of the prepared sausage at 49 C resulted in a progressive loss of virus. However, after 6 hr at 49 C, an average of 1.1×10^3 infectious units of virus per gram of sausage remained of an initial 7.5×10^5 infectious units per gram.

Studies in this laboratory *(1)* have shown that some enteroviruses are rapidly inactivated when exposed directly to growing cultures of proteolytic bacteria. However, the effect of microbial growth on survival of enteroviruses in foods has been studied only by indirect means. Lynt *(10)* tested the survival of several enterovirus types in a variety of foods, and concluded that decomposition which took place in foods stored at room temperature had no effect on virus survival. Only two of the food samples he tested showed marked bacterial growth during the storage time, pizza at 2.6×10^6 colonies per sample, and breaded shrimp, 2.9×10^6 colonies per sample (sample size not given). Kalitina *(7, 8)* experimentally contaminated autoclaved and non-autoclaved samples of mince meat and of cottage cheese with various enteroviruses, and found no significant difference in the persistence of virus in the paired samples. When survival times of enteroviruses in sterilized milk and in sour milk products were compared *(9)*, the temperature of storage and the fermentation process did not substantially influence the dynamics of virus inactivation. The effect of suppressing bacterial growth in enterovirus-contaminated spaghetti by use of tetracycline was reported by Cliver et al. *(3)*. The apparent decrease in putrefaction in the food sample containing the antibiotic did not enhance virus persistence.

Because of the lack of information on the effect that bacterial growth has on persistence of viruses in foods, we investigated the survival of a model enterovirus in two types of ground meat products: Thuringer sausage, which undergoes a defined, non-proteolytic fermentation during its preparation, and market ground beef, which is often contaminated with both proteolytic and non-proteolytic bacteria. Although neither sausage nor ground beef has been directly implicated in outbreaks of enterovirus caused disease, enteroviruses have been isolated from market samples of ground beef *(12)*. Coxsackievirus type A9 (CA 9) was selected as the model virus because it was more protease-sensitive than any of the other enteroviruses that we had tested.

MATERIALS AND METHODS

Virus and tissue cultures

Coxsackievirus type A9 (CA9), strain Bozek, was obtained from the American Type Culture Collection. Tissue cultures used to propagate and titrate this virus were primary Rhesus (*Macaca mulatta*) monkey kidney (PMK) monolayers. The procedures to prepare these cultures and their use in virus titration in our laboratory have been described *(2)*.

Sausage preparation and experimental contamination

Batter to prepare Thuringer sausage was obtained from Oscar Mayer and Co., Madison, Wis. The culture it contained was a *Lactobacillus* species. For determining virus survival, CA9 (10 ml of a stock virus suspension that had been extracted with ether and dialyzed overnight against 1 liter of distilled water) was added to 50 lb (22.7 kg) of sausage batter and mixed in by hand kneading. The batter was then stuffed into sausage casing, so that 10 or more sausages were obtained weighing about 3-4 lb (1.4-1.8 kg) each. After a 24-hr fermentation at 30 C, the sausage was heated at 49 C for periods up to 6 hr. The details of the apparatus and procedures used for the preparation of this sausage have been described *(4)*.

Determinations of *Lactobacillus* concentration were made by surface inoculation of 0.1 ml quantities on APT agar (Difco). Virus content was determined by blending 11 g of sausage in 99 ml phosphate-buffered saline (PBS) in a Waring blender for 1 min. The homogenate was centrifuged at $2000 \times g$ (max) for 10 min. Five milliliters of supernatant fluid was added to an equal volume of cold diethyl ether and mixed for 2 min on a Vortex mixer. After refrigeration for 30 min, samples were centrifuged 20 min at $2000 \times g$ (max). The aqueous layer was diluted in PBS plus 2% agamma calf serum for virus titration in PMK tissue cultures.

Experimental contamination of ground beef

For these trials, 15 ml of stock CA9 diluted 10^{-1} in PBS were added to 600 g of ground beef (chuck) and kneaded in by hand. Four 150-g patties were made; these were stored at room or refrigerator temperature for 2 weeks. Eleven-grams samples were taken at the initial time and at intervals for 2 weeks, and treated in the same manner as described for sausage above. Plate count agar (Difco) was used for

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bacterial enumeration; acidified potato-dextrose agar (Difco) was used for mold and yeast count determination.

RESULTS

Persistence of CA9 during sausage production

The results presented in Table 1 show that during the fermentation process at 30 C, approximately 85% of the input virus was lost. Heating the prepared sausage for 1, 3, and 6 hr at 49 C caused a progressive loss of virus. However, even after 6 hr of heating, an average of 1.1×10^3 plaque-forming units (PFU) of virus per g of sausage remained in the samples tested. The pH of the sausage before fermentation was 6.0; after fermentation, 4.8. The initial *Lactobacillus* concentration was 4.1×10^6 colonies/g. After fermentation, values for the four samples tested ranged from 7.0×10^8 to 1.5×10^9 colonies/g.

Persistence of CA9 in ground beef

Ground beef samples were stored either at room (23 C) or refrigerator temperature (4 C). At both temperatures, bacterial growth was rapid and reached high numbers per gram (Table 2). By 8 days, the total plate count was approximately the same at both temperatures for all samples. Of 5 different bacterial types, picked on the basis of colonial morphology, 3 from the samples that had been stored at room temperature were proteolytic. The concentration of molds and yeasts found in 4 days' incubation at both temperatures was so low ($< 10^3$ colonies per gram) that further counts were omitted.

At 4 C, 2 weeks were necessary before significant virus loss was noted. At 23 C, some loss occurred by 8 days' incubation, but 2 weeks were also required for a high degree of virus inactivation to occur.

TABLE 1. PERSISTENCE OF CA9 IN SAUSAGE PRODUCTION

Time	Temp (°C)	Sample No.	Virus concentration (PFU/g)	Mean PFU/g	% PFU remaining
Initial time	—	1	7.7×10^5		
		2	7.8×10^5		
		3	7.2×10^5	7.5×10^5	—
		4	7.4×10^5		
24 hr fermentation	30	1	8.0×10^4		
		2	7.0×10^4	1.2×10^5	16.0
		3	1.7×10^5		
		4	1.4×10^5		
+ 1 hr	49	1	3.1×10^4		
		2	5.0×10^4	3.2×10^4	4.3
		3	2.1×10^4		
		4	2.5×10^4		
+ 3 hr	49	1	1.6×10^4		
		2	1.3×10^4	1.5×10^4	2.0
		3	1.7×10^4		
		4	1.6×10^4		
+ 6 hr	49	1	1.1×10^3		
		2	1.1×10^3	1.1×10^3	0.1
		3	9.0×10^2		
		4	1.1×10^3		

TABLE 2. PERSISTENCE OF CA9 IN GROUND BEEF

Storage temp (°C)	Sample replicate	Storage time (days)	Bacterial colonies/g	Virus PFU/g	% PFU remaining
4	1	0	7.0×10^5	9.3×10^4	—
		2	5.6×10^8	6.1×10^4	66
		4	3.8×10^8	8.4×10^4	90
		6	2.0×10^9	7.6×10^4	82
		8	6.0×10^9	7.8×10^4	84
		14	9.5×10^8	1.9×10^2	0.2
	2	0	6.5×10^5	1.1×10^5	—
		2	5.7×10^8	5.2×10^4	47
		4	9.0×10^8	9.8×10^4	89
		6	5.1×10^9	1.0×10^5	91
		8	4.8×10^9	9.3×10^4	85
		14	7.1×10^8	$<1.0 \times 10^2$	<0.1
23[a]	1	0	7.0×10^5	9.3×10^4	—
		2	2.2×10^9	8.6×10^4	92
		4	5.9×10^9	8.8×10^4	95
		6	3.9×10^9	2.6×10^4	28
		8	2.8×10^9	3.0×10^4	32
		14	7.2×10^8	1.1×10^1	0.01
	2	0	6.5×10^5	6.2×10^4	—
		2	5.8×10^7	6.2×10^4	100
		4	6.8×10^9	5.8×10^4	94
		6	6.1×10^9	1.8×10^4	29
		8	4.6×10^9	1.3×10^4	21
		14	1.1×10^9	$<1.0 \times 10^2$	<0.2

[a]Room temperature

DISCUSSION

The persistence of virus in experimentally contaminated ground meats did not appear to be significantly affected by the presence of bacteria, at least for the relatively short periods of storage time used here. In ground beef, CA9 was not rapidly lost, even though proteolytic bacteria were present in high numbers. By the time loss of virus became rapid enough to begin to afford some protection to the consumer (after 8 days at room temperature), the meat could hardly have been considered edible; it had undergone extensive putrefaction. The virus losses observed between 8 and 14 days may have been proteolytic. In the preparation of sausage, CA9 was able to withstand both the bacterial fermentation process and subsequent heat treatment. From these studies, it appears that enteroviruses are quite stable in ground meat products, which are among the most likely foods to become contaminated with virus through human handling.

A very significant observation during these studies was the relatively uniform distribution of virus found in each of the samples, both in ground beef and in

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sausage. This suggests that contamination of foods under natural conditions would result in a similar pattern of virus distribution. Thus, a random sample taken from a suspected food should contain detectable virus, provided that the level of contamination was sufficiently high. From the data reviewed by Grabow *(5)*, the quantity of poliovirus excreted in feces during the carrier period averages 10^4 infectious units per gram. Thus, if 0.1 g was accidentally introduced into ground beef during the preparation of 100 beef patties (100 g each), the resulting concentration of 0.1 infectious unit per gram would be just below the sensitivity level for detection, based on current methodology *(6)*. The total number of infectious units per 100-g patty *(10)* could, however, be sufficient to cause infection *(11)*. At peak levels of enterovirus excretion, as many as 10^6 infectious units per gram of feces are present. Applied to the example given above, this quantity of virus would readily be detected, as well as being more than a sufficient quantity to initiate infection.

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