

SURVIVAL OF SALMONELLAE AND MYCOBACTERIA IN SALTED AND UNSALTED SWINE GUTS USED AS SAUSAGE CASING AND SAUSAGE EMULSION OF A HARD SALAMI

J. PALÁSEK, M. PAVLAS, Iva KUBŮ

Veterinary Research Institute, 621 32 Brno

Received August 24, 1990

Abstract

Palásek J., M. Pavlas, Iva Kubů: *Survival of Salmonellae and Mycobacteria in Salted and Unsalted Swine Guts used as Sausage Casing and Sausage Emulsion of a Hard Salami*. Acta vet. Brno, 60, 1991: 375–381.

Salmonellae survived at room temperature (18–22 °C) in salted swine guts used as sausage casing for 7–21 days. At cooling temperature (4 °C), *Salmonella typhimurium* was reisolated after revival from control unsalted guts still after 70 days, and in the salted ones even after 154 days. *S. enteritidis* was less resistant at 4 °C than *S. typhimurium*, surviving 28 and 126 days in unsalted and salted guts, respectively.

M. intracellulare and *M. avium* survived in unsalted guts 91 and 35 days, respectively. In salted guts, the survival of *M. avium* was 6 times longer compared with that in unsalted ones. The dynamics of mycobacterium survival indicate that in salted guts stored at cooling temperature, *M. avium* and *M. intracellulare* are killed within 6 and 12 months, respectively.

During the ripening of a hard salami (Poličan), processed without heat treatment, *S. enteritidis* was reisolated after revival even after 42 days, while, *S. typhimurium* was killed after 35 days already.

Swine, Salmonellae, mycobacteria, salted guts, ripening of salami

Salmonellae remain at the top of the list of alimentary infections for a long period and their high resistance against environmental effects has been well documented.

Sodium chloride inhibits the growth of salmonellae at a concentration of 7 % already, but rapid killing only occurs when the concentration reaches 30 % (Koelensmid and van Rhee 1964, cit. Morse and Duncan 1974, Walsh 1972). Temperature is also an important factor for the survival of salmonellae. It has been demonstrated that at 4–7 °C salmonellae survived in 10–30 % sodium chloride 106 days at least and that the survival time was 5 times shorter at 18–22 °C (Herler 1971, cit. Grieger 1978).

High resistance of *M. avium* to chemical and physical effects of the environment is well known (Pavlas and Dokoupil 1967). *M. avium* survived 1 year in the meal and 5 months in wheat and pea (Kiriljuk 1966). In manure where the conditions for survival of mycobacteria are less favourable, *M. avium* lost its virulence gradually and was killed after 2–3 months (Širobokova 1964).

At present, tuberculous lesions are found in the guts of approximately 0.3 % of pigs slaughtered in Czechoslovakia, their findings in parenchymatous organs being rather sporadic. The prevalence represents in the Czech Republic several tens of thousands slaughtered pigs per year, which are released for human consumption under strictly set conditions only. Data on the length of mycobacteria survival in swine guts used as sausage casing and on the effects of salting have not been reported.

Takacs and Simonffy (1970) investigated the survival of salmonellae in ripening salami processed without heat treatment and stated that salmonellae were killed in most cases and that their survival depended on the rate of contamination of the raw sausage emulsion. Complete killing did not occur when the count exceeded $2 \cdot 10^4 \cdot \text{g}^{-1}$.

In Czechoslovakia the survival of salmonellae in hard sausages was studied by Gayer (1978).

The aim of our experiments was to examine the survival times of salmonellae and mycobacteria in salted and unsalted swine guts stored at various temperatures and the fate of salmonellae during the ripening of a hard salami processed without heat treatment.

Materials and Methods

Mucosa was separated by hand from fresh swine guts, which were then treated with an amount of sodium chloride, corresponding to the original weight of fresh guts. The guts were cut into 20 cm long segments and one end was tied. Then 0.2 ml of 24 h broth culture of *S. typhimurium* or *S. enteritidis* was applied into each segment and the other end was tied. Control, unsalted guts were infected similarly. The guts were then stored at 18–22 °C or at 4 °C.

Samples of the gut wall weighing ca. 1 g were transferred at regular intervals into 10 ml of buffered peptone water. After 18 h incubation at 37 °C, 0.1 ml of the buffered peptone water was transferred into 10 ml Rappaport medium and 1 ml into 10 ml selenite medium. After another 24 h of incubation at 42 °C, the samples were inoculated onto agar with brilliant green and phenol red. The growth was evaluated and colonies counted after 24 h incubation. The incidence of salmonellae was confirmed by the rapid slide agglutination test with a polyvalent serum.

The killing effect of sodium chloride on *M. avium* and *M. intracellulare* was also examined in fresh mucosa-free guts, which were salted, as mentioned above. Ten cm long segments were infected into the inner side by suspension of mycobacteria, containing 1 mg of culture in 1 ml of sterile physiological saline and 0.5 ml of mycobacterial suspension was inoculated into each segment. Infected guts were stored at 4 °C. Unsalted guts, infected in the same way, were used as a control. Mycobacteria were detected in guts after rinsing in sterile distilled water. The guts were then cut lengthwise and dipped into sterile distilled water for 15 min. After maceration of guts, the liquid was poured off, centrifuged and the sediment was prepared with HCl and NaOH. After decontamination, the sediment was cultured on solid egg medium and fluid Šula medium. The culture media were incubated at 37 °C and evaluated in 1–2-week intervals during 2 months.

The sausage emulsion for a herd salami "Poličan" was obtained from a meat plant in Kroměříž. After infection with broth culture of *S. typhimurium* or *S. enteritidis* (10^8 cells per 1 g of sausage emulsion), the emulsion was filled into original cover and stored hanging in an air-conditioned box "MYTRON" under the conditions valid for this type of salami (Table 1). Humidity was measured with a hygrometer. Samples for salmonella detection were taken in a week intervals and were treated similarly as those of infected swine guts.

Results

The initial *S. typhimurium* concentration was 10^7 . g⁻¹ in both salted and unsalted guts (Table 2) at room temperature 18–22 °C. The control showed at first increase of cells up to 10^9 . g⁻¹, but a rapid decrease occurred after 7 days, and after 21 days salmonellae were re-isolated only after revitalization. The decrease of *S. typhimurium* count was even more rapid in salted guts and after 14 days salmonellae were detected even after revitalization.

Table 1

Storing conditions of the sausage emulsion for hard salami "Poličan" infected with salmonella

Period day	Temperature °C	Relative humidity %
0–14	25	85–90
15–21	22	85
22–28	19	80
29–35	16	75
• 36–42	13	70

The initial *S. enteritidis* concentration was lower (10^6 . g⁻¹). Increase up to 10^9 . g⁻¹ was recorded at first in unsalted guts, similarly as in *S. typhimurium*, then the concentration decreased and after 28 days no salmonellae were re-isolated even after revitalization. In salted guts *S. enteritidis* was killed within 14 days and no salmonellae were re-isolated later.

At the cooling temperature (Table 3) *S. enteritidis* survived shorter period and was isolated within 28 and 126 days in unsalted and salted guts, respectively.

Mycobacteria survived much longer in swine guts, compared with salmonellae. *M. intracellulare* (Fig. 1), which is more resistant, survived up to 13 weeks, however *M. avium* was killed after 5 weeks. In salted guts the survival of mycobacteria was longer. The survival period in *M. avium* (Fig. 2) was nearly 6 times longer (30 weeks) compared with unsalted guts. *M. intracellulare* survived as long as 7 months in salted guts, but the number of vital ones decrease to about 1/3.

Differences were recorded between the two salmonellae serotypes tested for their survival in the sausage emulsion (Table 4). The number of *S. typhimurium* decreased after 21 days so that they were detectable only after revitalization and after 35 days no salmonellae were reisolated.

Table 2
Survival of salmonellae in salted and unsalted guts at 18–22 °C

Day	<i>S. typhimurium</i>		<i>S. enteritidis</i>	
	salted	unsalted	salted	unsalted
0	10^7	10^7	10^6	10^6
1	10^7	10^6	10^7	10^6
3	10^6	10^6	N	N
7	10^4	10^6	10^6	10^6
14	—	10^4	10^5	10^5
21	—	+	—	10^5
28	—	—	—	—
35	—	—	—	—

The figures show salmonellae counts per 1 g
+ . . . positive after revitalization
N . . . not examined

Table 3
Survival of salmonellae in salted and unsalted guts at +4 °C

Day	<i>S. typhimurium</i>		<i>S. enteritidis</i>	
	salted	unsalted	salted	unsalted
0	10^7	10^7	10^6	10^6
1	10^6	10^7	N	N
3	10^7	10^6	N	N
7	10^7	10^6	10^7	10^6
14	10^6	10^6	N	N
21	10^6	10^6	10^7	+
28	10^5	10^4	10^6	+
35	10^4	10^3	10^6	—
42	+	+	10^5	+
49	+	+	10^5	+
63	+	+	+	+
70	+	+	+	+
126	+	—	+	+
154	+	—	—	—

The figures show salmonellae counts per 1 g
+ . . . positive after revitalization
N . . . not examined

Table 4
Survival of salmonellae during the ripening of hard salami "Poličan"

Days	<i>S. typhimurium</i>	<i>S. enteritidis</i>
0	10 ⁸	10 ⁸
7	10 ⁸	10 ⁸
14	10 ⁸	10 ⁸
21	+	10 ⁸
28	+	10 ⁸
35	-	+
42	-	+

The figures show salmonellae count per 1 g
+ . . . positive after revitalization

S. enteritidis was more resistant and was detected by direct culture still after 28 days. This serotype was detectable 42 days after revitalization, which is the period necessary for ripening of the hard salami Poličan.

Discussion

The survival of salmonellae in guts infected both naturally and artificially and stored in a saturated solution of NaCl (pH 4.0 and 10.0) at 6 °C was studied by Gabis and Siliker (1974). Under these conditions, salmonellae were killed already after 24 h. If the guts were placed into crystalline sodium chloride, salmonellae were killed after 7 and 21 days in sheep and swine guts, respectively.

Our results demonstrated markedly longer survival periods, especially at cooling temperature, when *S. typhimurium* survived after revitalization 154 and 70 days, in salted and unsalted guts, respectively. *S. enteritidis* was less resistant under the same conditions and survived 126 and 28 days, respectively.

Long survival of both the serotypes of salmonellae in salted guts demonstrate

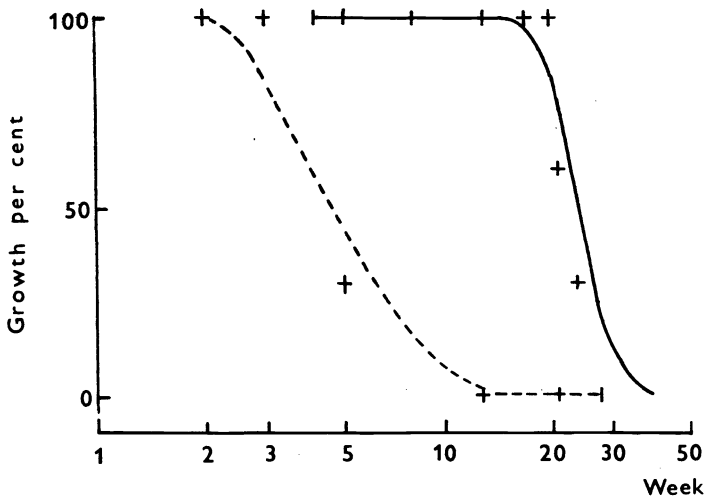


Fig. 1. Survival of *M. intracellulare*

their great resistance against high concentrations of sodium chloride in the medium.

In salted guts infected with mycobacteria, autolytic influence is apparent with the growth of accompanying psychotrophic microflora. The dynamics of mycobacteria killing shows that in salted guts *M. avium* survives six months and more and *M. intracellulare* even 1 year and more. The data indicate a great resistance of mycobacteria causing the infection of pigs.

The initial salmonellae count is important for their survival in a salami processed without heat treatment. Their killing is explained by the decrease of a_w and pH. Takacs and Simonffy (1970) reported that killing of salmonellae is also influenced by catalase activity suppression by produced hydroxylamine. The accumulated hydrogen peroxide is destroying the cells of microorganisms, including salmonellae. Addition of a starting culture to the sausage emulsion of salami processed without heat treatment might significantly shorten the survival time of salmonellae (Gayer 1978). A long survival of salmonellae in another type of hard salami was recorded by Bartas (1982). He re-isolated salmonellae after revitalization 80 days following processing, though the salami was of a very hard consistence. It demonstrates great resistance of salmonellae against drying and decrease of water activity.

Practically it means that salmonellae count hardly exceeds $10^3 \cdot g^{-1}$ in the sausage emulsion for hard salami "Poličan". If for all that such a number appears, salmonellae would not be killed during the ripening of salami.

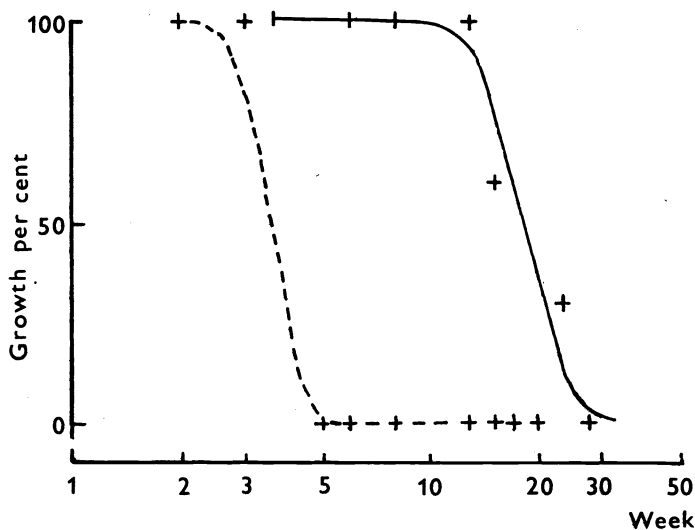


Fig. 2. Survival of *M. avium*
 medium with NaCl ————
 without NaCl - - - - -

Přežívání salmonel a mykobaktérií v solených a nesolených vepřových střevech a díle trvanlivého masného výrobku

Při pokojové teplotě (18–22 °C) v solených střevech přeživaly salmonely do 7.–12. dne. Při chladírenské teplotě (+4 °C) *Salmonella* (*S.*) *typhimurium* byla z kontrolních nesolených střev zachycena po resuscitaci ještě 70. den, u solených

dokonce po 154 dnech. *S. enteritidis* při chladírenské teplotě byla méně odolná ve srovnání se *S. typhimurium*, přičemž v nesolených střevech byla zachycena pouze do 28 dnů; v solených střevech vydržela 126 dnů.

Při posouzení devitalizačního účinku chloridu sodného na *Mycobacterium avium* a *M. intracellulare* při chladírenské teplotě přežívalo *M. intracellulare* v nesolených střevech 91 dní, *M. avium* 35 dní. U solených střev probíhala devitalizace mykobaktérií daleko pomaleji. Doba přežívání *M. avium* byla 6× delší ve srovnání s nesolenými střevy. Z dynamiky přežívání mykobaktérií vyplynulo, že v nasolených střevech se dá předpokládat devitalizace *M. avium* za půl roku, u *M. intracellulare* až za 1 rok po jejich uložení v chladírenské teplotě.

Sledováním přežívání salmonel při zrání tepelně neopracovaného masného výrobku — trvanlivého salámu typu Poličan byla zjištěna větší odolnost u *S. enteritidis*, která byla izolována po resuscitaci i po 42 dnech na rozdíl od *S. typhimurium*, která byla devitalizována již po 35 dnech.

Выживание сальмонелл и микобактерий в условиях повышенного содержания хлорида натрия

Исследовали способность сальмонелл и микобактерий комплекса *M. avium — intracellulare* выживать в соленой кишке свиньи и влияние технологии производства на выживание сальмонелл у теплом необрабатываемых мясных продуктов типа Поличан. При комнатной температуре выживали сальмонеллы в соленой кишке до 28-ого дня. Температура холодильника позволяла выживание сальмонелл выше 5 месяцев. *S. typhimurium* изолировали из контрольной несоленой кишки после ресусцитации еще 70. день, из соленой даже после 156 дней. *S. enteritidis* была в температуре холодильника менее устойчива в сравнении с *S. typhimurium*, ее изолировали в несоленой кишке только до 28-ого дня.

Исследованием выживания сальмонелл на протяжении созревания геплом необработанного мясного продукта — салами длительного хранения типа Поличан — определили высшую устойчивость у *S. enteritidis*, которую изолировали после ресусцитации через 6 недель, в сравнении с *S. typhimurium* которая погибала до 5 недель.

При оценке действия хлорида натрия на *M. avium — intracellulare* в температуре холодильника докладывали, что *M. intracellulare* выживало в несоленых кишках до 13 недель, *M. avium* 5 недель. В соленых кишках происходило разрушение микобактерий значительно медленнее. Срок выживания *M. avium* был шесть раз дольше чем у несоленой кишки. Из динамики выживания микобактерий стало ясно, что в соленых кишках можно предполагать гибель *M. avium* через пол года, гибель *M. intracellulare* через год при их хранении в температуре холодильника.

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