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The Behaviour of Salmonellae in Vacuum-Packaged Cooked Cured Meat Products

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Introduction

It is well known that the systems used for reporting food-borne illness are quite inadequate. In the U.K. for example, where the reporting procedure is considered superior to most, it has been noted (Dr. B.C. Hobbs, personal communication) that with Salmonella food poisoning the food vehicle was identified in only 1% of family outbreaks and 16% of general outbreaks (1968 figures). Of the remainder, who can say which foods were or were not involved? Despite these shortcomings, the available data indicate that vacuum-packaged ready-to-eat cured meats have seldom been associated with salmonellosis. Perhaps as a consequence of the apparent low "Salmonella risk", there have been few published studies on the behaviour of salmonellae in such products.

Under the Canadian Food and Drugs Regulations vacuum-packaged meats must be held under refrigeration at all stages during storage, transportation and sale. In our experience, this is not always adhered to. For this reason, and due to the lack of information, we have investigated the fate of salmonellae in a selection of vacuum-packaged meat products held under abusive conditions, and attempted to assess concomitant public health hazards.

Experimental

Meat Products. Six ounce (c 170 g) packages of sliced mock chicken, bologna and ham, and 8 ounce packages of wieners were obtained from normal production immediately after vacuum packaging.

Salmonella Inoculation, and Analysis of Stored Products. The packages were cleaned on one side with alcohol, and a rubber gasket (c 1.5 cm diameter) glued to the surface. One ml of an appropriate dilution (in 0.1% peptone water) of a 24 hour nutrient broth culture of Salmonella typhimurium* was then injected through the gasket into the meat. The dilutions were chosen to give three initial levels of salmonellae, i.e. approximately 10, 100, and 1000 per g of meat. Control samples were inoculated with 1.0 ml of sterile peptone water.

Inoculated packages of mock chicken were held at 7, 24, and 37°C. It was later decided that 37°C was unrealistically high, and thus with the other products, the storage temperatures were 7, 18, and 24°C.

Packages were removed at intervals during storage and first assessed for appearance and odour. Numbers of salmonellae were estimated using a 3-tube Most Probable Number technique.

* Strain supplied by Dr. H. Pivnick, Canada Department of National Health and Welfare.

Contents of the package were blended for 2 minutes (Waring Blendor) with an equal weight of sterile distilled water. In the case of wieners the contents (8 ounces) were comminuted and a 6 ounce sample taken and blended as above. One ml of each of three consecutive decimal dilutions of homogenate were distributed into tubes (3 per dilution) of Selenite Cystine Broth.

After incubation for 24 hours at 37°C, a loopful from each tube was streaked on Brilliant Green Agar (Difco). Plates were incubated at 37°C for 24 hours and presumptive Salmonella colonies were confirmed with polyvalent antisera.

The total viable count in each sample was estimated by a pour-plate method using Plate Count Agar (Difco). Colonies were enumerated after incubation at 25°C for 3 days. With mock chicken only, the composition of the microflora during storage was determined. Colonies were randomly removed from total count plates and grouped according to the following characteristics: morphology, motility, Gram reaction, oxidase, catalase and oxidation/fermentation test.

Results and Discussion

Before discussing our findings, it should be borne in mind that due to the inherent inaccuracies of the MPN method, the data on Salmonella numbers are approximate.

Mock Chicken. The behaviour of salmonellae in mock chicken stored at 7, 24 and 37°C is shown in Figures 1a, b and c respectively. At 7°C storage, with an inoculum of

100 or 1000/g, the salmonellae persisted for several weeks. When 10/g were present initially, salmonellae were not detected after 2 weeks. These results do not necessarily mean that no salmonellae were present after prolonged storage, since it is possible that cells were still viable, but physiologically injured and therefore unable to recover in the selenite enrichment broth.

Growth of salmonellae occurred at all three inoculation levels in samples held at 24°C (Figure 1b). For example, at 3 days and an inoculum of 1000/g, salmonellae increased more than ten thousandfold to about 10^7/g (i.e. a total of $>10^9$/170 g package). Although the "minimal infective dose" is not known, numbers considerably less than 10^9 have reportedly caused illness (National Research Council. Committee on Salmonella, 1969).

It is noteworthy that samples containing high numbers of salmonellae were judged to be organoleptically acceptable. Apart from moisture accumulation in the package and slight colour fading (both of which occurred in inoculated and control samples) no other abnormalities in appearance or odour were noted.

Salmonella multiplication was most rapid in samples held at 37°C, and levels of between 10^7 and 10^8/g were achieved regardless of initial numbers (Figure 1c). Again the product was organoleptically acceptable.

Studies on the microflora of mock chicken showed that, prior to storage, several groups of bacteria including lactic acid bacteria, Pseudomonas, Acinetobacter, microbacteria and coliforms were present. At this stage, the total count on control samples was low (<100/g). The organisms comprising this count either had survived the heat treatment given to the product, or were post-processing contaminants. The latter would seem to be the more likely source, since the product is heated to an internal temperature of 70°C, and rough calculation of the total lethality of the process indicated that most non-sporing species would be destroyed.

During storage, at all three temperatures, the microflora became dominated by lactic acid bacteria. Significantly, salmonellae were isolated from total count plates derived from certain samples and corroborated results obtained with the MPN method.

The fact that salmonellae grew in mock chicken is not really surprising if one compares the physiological characteristics of salmonellae with the relevant properties of the meat product. Thus it can be seen (Table 1) that the O_2 tension, pH, water activity (a_W), nitrite and salt concentrations of mock chicken (and the other products studied) are all within the limits for Salmonella growth.

Some of the data given in the table warrants further comment. The minimum inhibitory concentration (mic) of nitrite for salmonellae, i.e. 800 to 4000 ppm, is for organisms in a medium at pH 6.7 (Castellani and Niven, 1955).

The pH of most cured meats is below this, and since the antibacterial activity of nitrite is pH-dependent, the mic for salmonellae in meats would be considerably less than the values shown. The thermal resistance is expressed in terms of decimal reduction times at 60°C. However, D values will be affected by other environmental factors such as salt concentration and a_w. These are only examples of the many complex interrelationships which can occur and therefore it would be inadvisable to take any of the data in Table 1 out of context.

It will be seen that the thermal process given to cooked meats is at least equivalent to 100 minutes at 60°C. Salmonella including heat resistant strains, eg S. senftenberg 775W, would almost certainly be eliminated by such a process. For a problem to exist, salmonellae would have to be acquired after processing, either in the packinghouse or in the home.

Bologna. Results obtained with bologna are summarized in Figures 2a to 2d. As with mock chicken salmonellae were recoverable from samples stored for several weeks at 7°C (Figure 2a). It will be seen from Figure 2b that the Salmonella counts in 18°C samples were extremely erratic. Although it is difficult to interpret the data, the results suggest that some multiplication occurred. For example, with the highest inoculum (1000/g), more than 10^6/g were present at 2 weeks' storage. In samples held at 24°C (Figure 2c) growth of salmonellae was considerably slower than in mock chicken held at the same temperature.

The reasons for this are not known but may be related to differences in product formulation and/or processing.

The growth of salmonellae in vacuum-packaged bologna was also studied by Goepfert and Chung (1970). Samples were inoculated with S. typhimurium or S. anatum at an initial load of 10^2 to $5x10^3$/g. During a 48-hour period at room temperature (23°C), numbers of both serotypes increased some 30-fold. These are much faster growth rates than those obtained in the present work, although they do compare with our findings on mock chicken (Figure 1b). Once again, these differences could be related to variations in manufacturing processes. In addition, the particular Salmonella strain used, the physiological state of the culture, the suspending fluid and method of inoculation may also influence subsequent growth patterns.

Ham. Figure 3a to 3d show the results obtained with ham. Samples stored at 7°C (Figure 3a) contained viable salmonellae even at 11 weeks. At 18°C (Figure 2b) with an inoculum of 10 or 100 salmonellae/g, the results were inconclusive and similar to those found with bologna. Thus the Salmonella count increased during the first 10 days or so, and then decreased. The apparent rise in count at 4 weeks could be attributed to variation between samples. When 1000/g were present initially, salmonellae reached numbers approaching $5x10^6$/g within 1 week. The count remained at this level for the next 2 weeks, then increased to $>10^7$/g.

Rate of growth at 24°C (Figure 3b) was inexplicably slow, and numbers barely increased during the first week even at the highest inoculum. Following this prolonged lag, fairly rapid multiplication took place during the next few days, and at 10 days salmonellae numbered about 10^6/g.

Wieners. Irrespective of storage temperature or inoculation level, salmonellae grew poorly if at all (Figures 4a to 4c). Some growth occurred at 24°C (Figure 4c), but to a maximum count of only $\leq 10^4$/g. Subsequently, a decline in numbers was noted.

The ultimate pH of wieners held at 18 and 24°C was 4.7 or 4.8. With ham the value was near 7.0, and with bologna and mock chicken, about 6.0. It is conceivable that the lower pH of wieners was responsible for the poor growth of salmonellae. Especially in the presence of salt, decreasing pH values are well known to have marked inhibitory effects (Alford and Palumbo, 1969).

Conclusion

It should be noted that the inoculation levels used in the present work were probably in excess of those occurring in naturally contaminated products. Nonetheless, we can conclude from the above data that a Salmonella hazard may arise when certain vacuum-packaged meat products are held at abusive temperatures for several days. It is difficult to assess the actual food poisoning risks since we do not know how often products become contaminated, nor the likelihood of contaminated products being stored for long periods at abusive temperatures.

Acknowledgements

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ble 1. Comparison of properties of salmonellae and cooked cured meats

Property	Salmonellae	Cooked Cured Meats
O_2 relationships	facultative anaerobe	vacuum packaged
pH	minimum for growth, 4.5[a]	initial value, 5.5 to 6.5
nitrite	mic at pH 6.7, 800 to 4,000 ppm[b]	50 to 100 ppm (after processing)
salt	inhibitory conc., 8.0%[c]	4.0% (water phase)
water activity	minimum for growth, 0.94[a]	$\underline{c}$ 0.98
heat resistance	D_{60^o} value, $\underline{c}$ 0.2 to 10 mins.[d]	F_{60^o} value >100 mins.

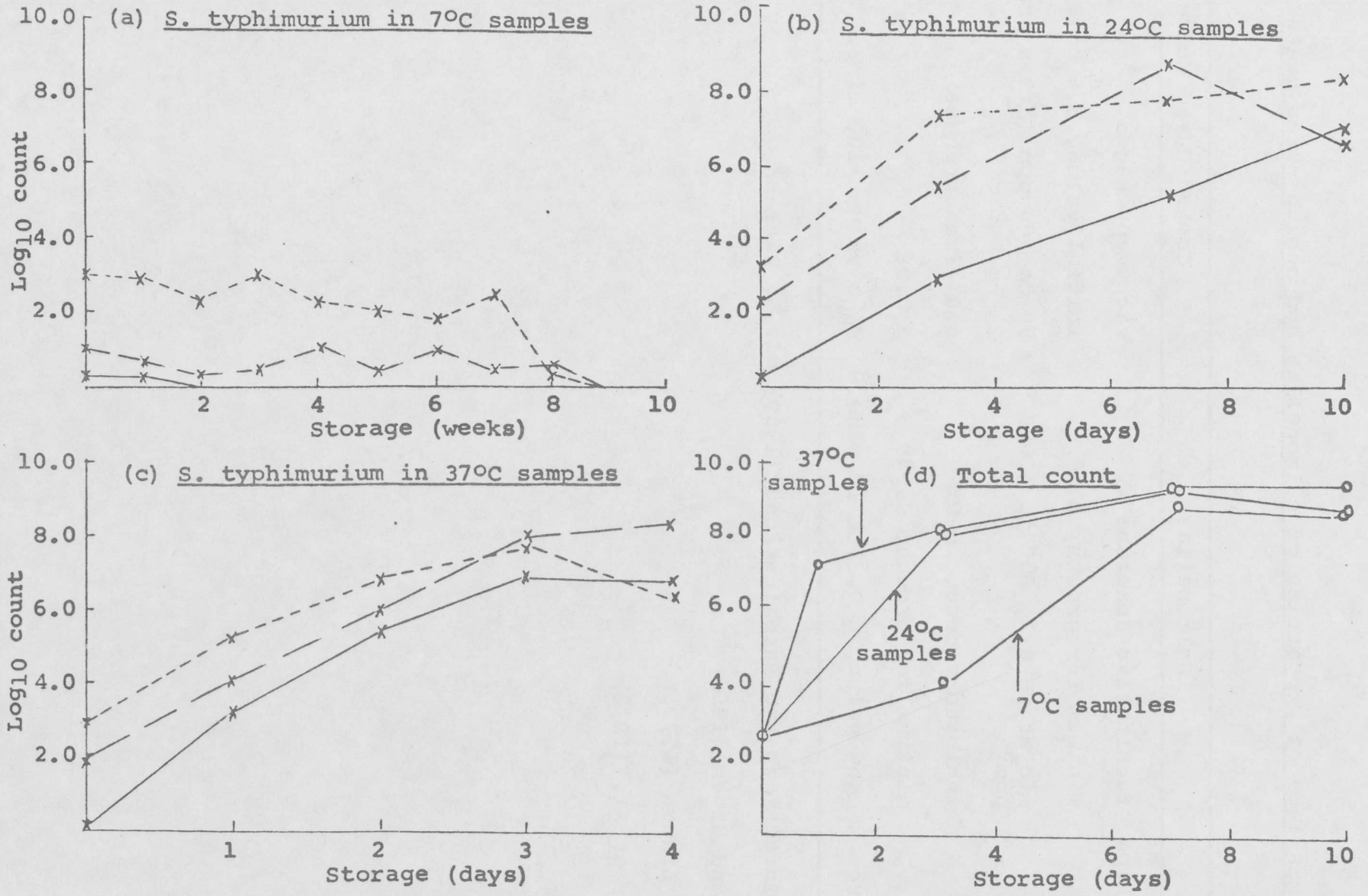
[a]Food-Borne Infections and Intoxications (1969) ed. H. Riemann

[b]Castellani and Niven (1955)

[c]Matches and Liston (1972)

[d]Baird-Parker et al. (1970)

Fig. 1. Behaviour of S. typhimurium, and changes in total count in vacuum-packaged mock chicken stored at different temperatures



Key

x——x inoculum 10 salmonellae/g
x— —x inoculum 100 salmonellae/g
x----x inoculum 1000 salmonellae/g

Fig. 2. Behaviour of S. typhimurium, and changes in total count in vacuum-packaged bologna stored at different temperatures

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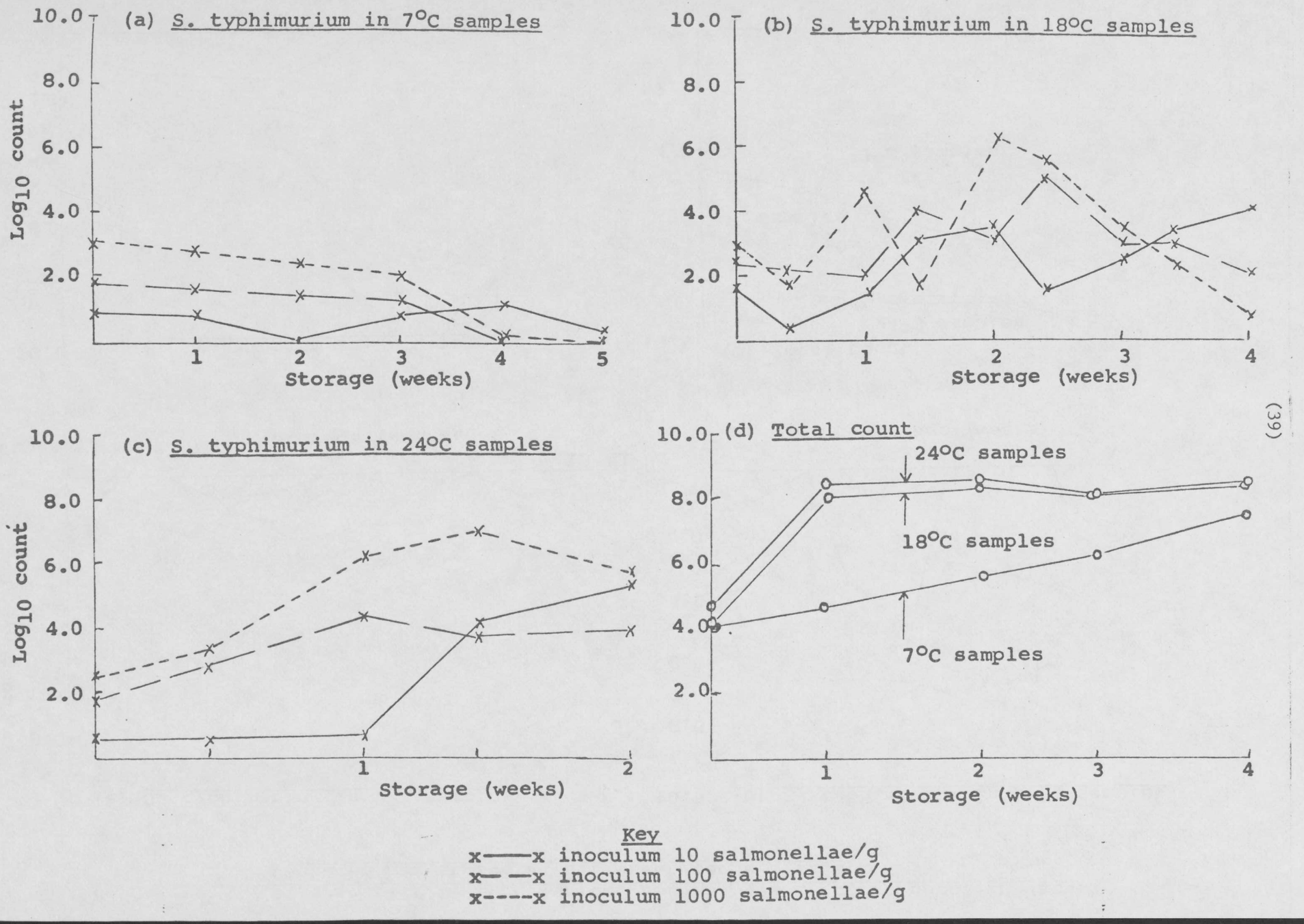


Fig. 3. Behaviour of S. typhimurium, and changes in total count in vacuum-packaged ham stored at different temperatures

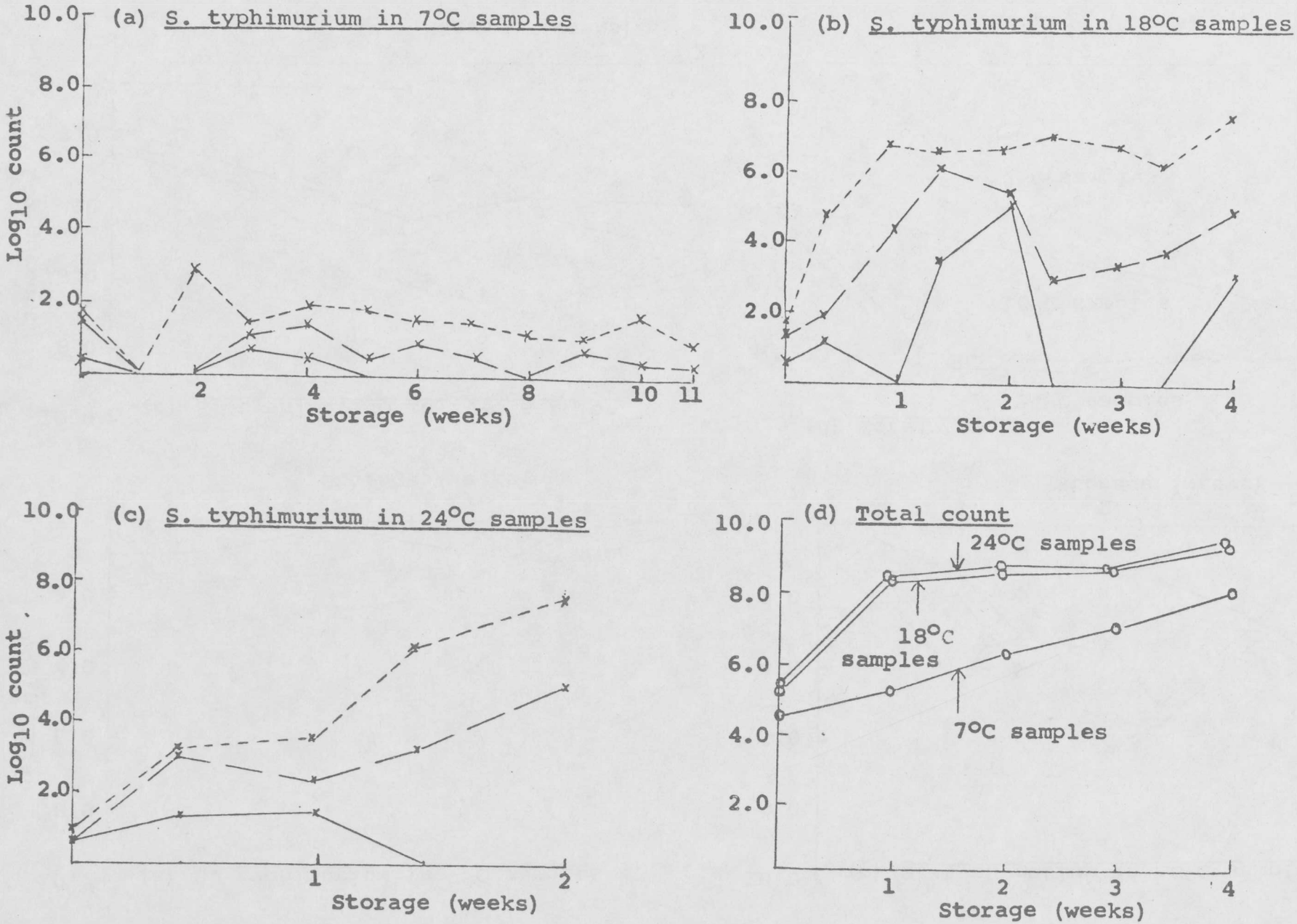
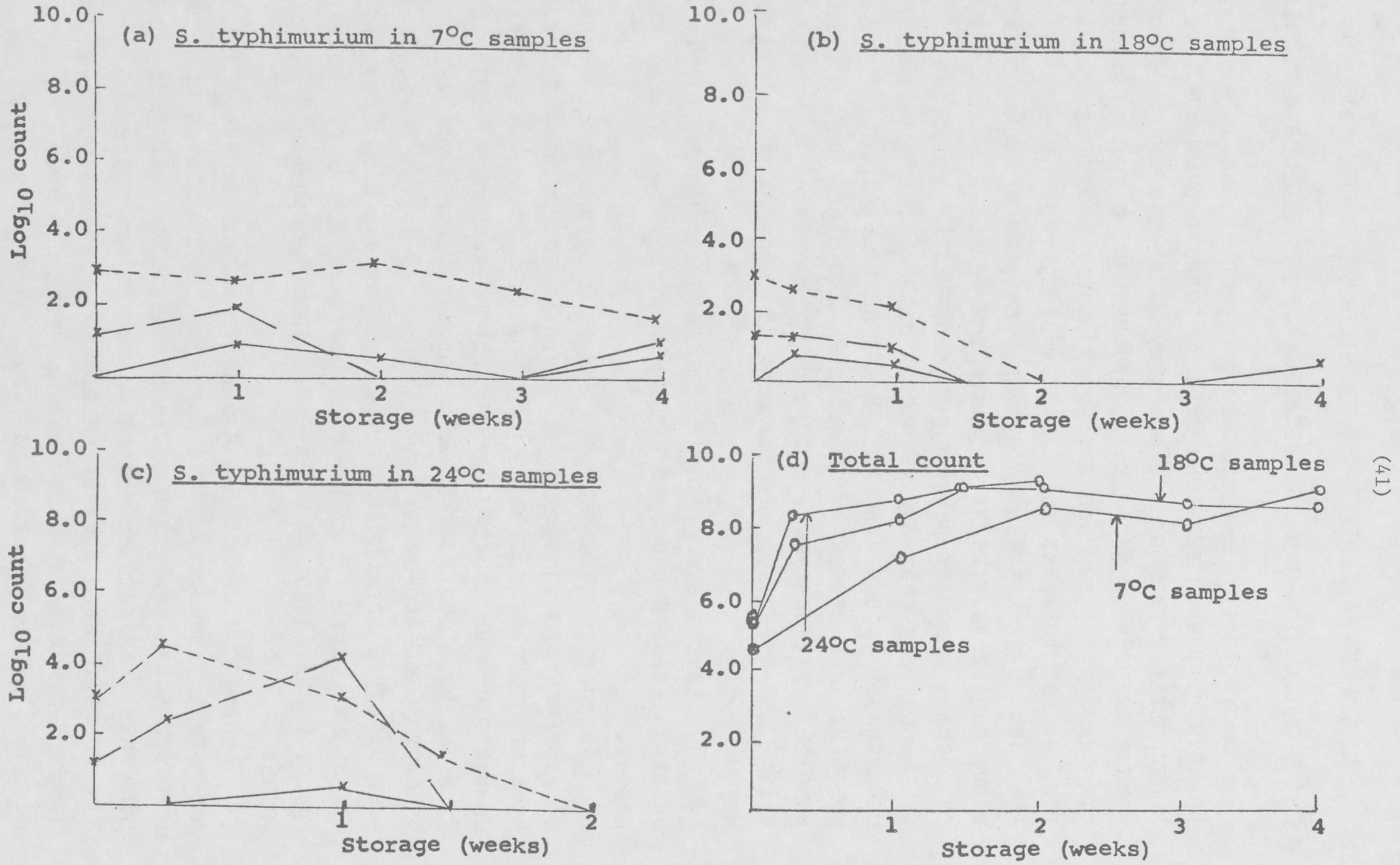


Fig. 4. Behaviour of S. typhimurium, and changes in total count in vacuum-

x----x inoculum 1000 salmonellae/g

Fig. 4. Behaviour of S. typhimurium, and changes in total count in vacuum-packaged wieners stored at different temperatures



Key

x——x inoculum 10 salmonellae/g
x— —x inoculum 100 salmonellae/g
x----x inoculum 1000 salmonellae/g