

PENETRATION RATE OF PROTEUS ORGANISM THROUGH EGG SHELL MEMBRANES AT DIFFERENT TEMPERATURES

A. R. AL Aboudi, I. M. S. Shnawa¹,
A. A. Hassen and R. B. Al-Sanjary

Department of Animal Hygiene and
Department of Microbiology¹,
College of Veterinary Medicine,
University of Mosul, Mosul, IRAQ.

ABSTRACT

Penetration of *Proteus* through shell membranes and egg contents were studied at two different temperatures of 4 and 25 °C and under a limited humidity level of 85-88%. *Proteus* cells survived on egg shell for 24 days and reached the yolk within six days and within twenty four days at 4 °C. The significance of these findings in egg spoilage and storing conditions have been discussed.

INTRODUCTION

Microbes affect egg contents by penetrating through the shell and inner membranes and can multiply to unacceptable levels causing egg rotting (Board, 1968). The principle microorganisms which affect eggs are Gram negative motile rods (Moat, 1960).

The reported factors affecting bacterial penetration rate are, shell quality, egg age, level of microbial contamination, bacterial species and storing temperature and humidity (Elliott and Brant, 1957; Hartung and Stadelman, 1968; Sauter and Peterson, 1974). However, most of these studies have been done either with *Salmonella* or *Pseudomonas* (Simmons *et al.*, 1970; Sauter and Peterson, 1974).

A recent study on egg microbial load has shown that *Proteus* species constitutes a high percentage among the Gram negative organisms contaminating egg shells and contents (Alaboudi et al., 1988). *Proteus* species may play a role in egg spoilage because of their motility, proteolytic and psychrophilic characters (Moursy et al., 1982).

This study was planned to evaluate the effect of storing temperatures on penetration by *Proteus* species through different egg structures.

MATERIALS AND METHODS

Fiftyfour fresh eggs were brought from Badoosh Poultry Plant. These eggs were cleaned and swabbed with 2% tincture iodine solution and dried on filter paper at room temperature for 2 - 3 minutes under aseptic conditions.

The eggs were divided into nine groups of six each. Four eggs of each group were immersed in *Proteus* cell suspension (37 °C) of 10⁸ cell per ml of nutrient broth for thirty seconds. After drying, the challenged and unchallenged (control) eggs of all groups were divided into two parts of 18 challenged and 9 control eggs each for incubation at 4 and 25 °C.

The eighteen challenged eggs incubated at both temperatures were distributed, among nine separated sealed containers containing 100 ml of saturated potassium chloride solution for each incubation temperature. Similarly, control eggs were also distributed in other separate containers as per test group.

At the designed periods of 0, 3, 6, 9, 12, 15, 18, 21 and 24 days, two challenged and one

control eggs at each incubation temperature were tested for the presence of *Proteus*. External and internal membrane side of the shell and yolk contents were swabbed with sterile wet cotton swabs moistened with nutrient broth. Swabs were inoculated into five ml nutrient broth and incubated at 37 C for hours before being streaked on MacConkey agar to observe *Proteus* growth.

RESULTS

Proteus showed penetration potential to hens egg shell and membranes. This penetrability was temperature dependent. Variable rates of penetration were noted among eggs stored at the same temperature and examined at the same time. The results are presented in Table 1.

Table 1: Penetrability and survival of *Proteus* organisms in hen's eggs stored at 4 C.

Incubation Period (days)	Presence of <i>Proteus</i>	
	25 C	4 C
0	Shell	Shell
3	Shell	Shell
6	Shell, inner membrane	Shell, inner membrane
9	Shell, inner membrane, yolk	Shell, inner membrane
12	Shell, inner membrane, yolk	Shell, inner membrane
18	Shell, inner membrane, yolk	Shell, inner membrane

21	Shell, inner membrane, yolk	Shell, inner membrane
24	Shell	Shell, inner membrane, yolk

At 25 °C storing temperature, the organisms penetrated the egg shell and shell membranes and reached yolk within six days of incubation. The organisms survived on shell, shell membrane and yolk for 21 days; however, at 24 days the proteus organisms were not isolated from the yolk or shell membranes.

At 4 °C the organisms penetrated the shell membranes within nine days and reached the yolk after 21 days. At the end of the incubation period (24 days) the organisms were isolated from the three different structures.

Control eggs at both 4 °C and 25 °C were free from bacterial growth throughout the experiment.

Neither signs of putrefaction nor spoilage could be detected throughout the experiment. However, physical changes in the yolk or white consistency were obvious in the eggs after three weeks storage at 25 °C.

DISCUSSION

The cuticle layer, the shell and shell membranes of eggs hold back most contaminating bacteria from reaching egg content of freshly laid eggs. Inner shell membrane is the most important physical barrier to contamination, followed by shell and outer shell membrane (Lifshitz *et al.*, 1964). Shell structure in contrast to shell thickness has an important affect on penetration rate (William *et al.*, 1968).

The study of survival on different egg spoilage organisms and the time needed to reach egg contents under different conditions are very important in order to evaluate the hygienic measures necessary to prevent egg deterioration. Concerning the spoilage organisms, most studies have been done with Pseudomonas species. Proteus species are well known to cause black rot spoilage of eggs (Jay, 1978).

Under the experimental conditions of relatively high moisture of 85-88% as provided by saturated potassium chloride solution (Rizk et al., 1966) and a temperature of 4 and 25 °C, Proteus species can survive on egg shell for upto 24 days. The storing temperature exerts a marked effect on penetration rates of Proteus through shell membranes. It took four to six days for the organisms to pass the shell membranes at 25 °C and nine days at 4 °C. The organisms thus reached the yolk within nine days at 25 °C, while it took more than three weeks at 4 °C. The variable rates of penetration within duplicated eggs are probably related to difference in egg shell quality (Hartung and Stadelman, 1963).

The used bacterial load of 10 cell per ml lies within the normal expected level of egg contamination. Ahmed et al., 1983 reported the presence of a mean count of 7×10^7 organisms on unwashed egg shell.

No sensory signs of spoilage were detected throughout the experimental period. This observation might be explained by the fact that Proteus have to multiply to acceptable spoilage levels before exerting its deteriorating effects.

Further studies with other spoilage organisms and under different storage conditions are necessary in understanding bacterial egg spoilage.

نفاذية جرثومة المتقلبات خلال العشية البيض
في درجتي الحرارة ٤ و ٢٥ م.

ابراهيم محمد سعيد شناوة ، اكرم ريشان العبودي
رعد بشير السنجري ، عبد الواحد احمد حسن

الخلاصة

درست معدلات نفاذية جرثومة المتقلبات خلال
العشية ومحتويات البيض في درجة حرارة خزن ٤ و
٢٥ م وتحت مستوى رطوبة محددة بـ ٨٥ - ٨٨ % .

استمرت الجراثيم بالحياة لفترة ٢٤ يوم على
القشرة ووصلت التي صفار البيض خلال ستة
ايام عند درجة حرارة ٢٥ م وخلال اربع وعشرون يوم
عند درجة حرارة ٤ م .

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