Study of microflora on egg shells in egg production in Jordan

Jenni Nordenskjöld

Uppsala BioCenter
Department of Microbiology
Faculty of Natural Resources and Agriculture Sciences
Swedish University of Agricultural Sciences

Uppsala

Independent project 2010:9

ISSN 1101-8151
ISRN SLU-MIKRO-EX–10/9–SE

Study of microflora on egg shells in egg production in Jordan

Jenni Nordenskjöld

Supervisor: **Hans Jonsson**
Examiner: **Stefan Roos**

Keywords: ***Jordan, egg shells, contamination, microorganisms, regulations***

EX0426 Independent project/degree project in Food Science
Bachelor C, 15 HEC within the Agriculture programme – Specialisation in Food Science

Uppsala BioCenter
Department of Microbiology
Faculty of Natural Resources and Agriculture Sciences
Swedish University of Agricultural Sciences

Uppsala

Independent project 2010:9

ISSN 1101-8151
ISRN SLU-MIKRO-EX–10/9–SE

PREFACE

The rise to this study is due to a Swedish organization called SIDA, the aim of SIDA is relief effort and to support developing countries with foreign aid (SIDA.se). SIDA also gives scholarships to students conducting studies in developing countries, so-called Minor Field Studies. I was granted one of these scholarships and have conducted a study in Jordan regarding the bacterial microflora on egg shell. The main objectives of the study has been changed a few times but the original purpose is almost the same as when the idea of studying the egg production in Jordan came to us from Dr Hamzah Al-Qadiri at Department of Agriculture, University of Jordan in Amman. Since Sweden have a highly developed control system for food safety and hygiene in a number of areas the idea of a study regarding the safety of eggs in Jordan rose. When the scholarship was granted the plans of this study started and fell into the thesis presented below.

Ultuna 3/6/2010

ABSTRACT

The title of this thesis is: Study of contamination factors on egg shell by microorganisms.

Egg production in Jordan is dominated by two large companies, but several smaller producers also sell their egg to the market. In this bachelor thesis the egg production in Jordan is studied with the aspect of the bacterial microflora on the egg shell. Eggs directly from farms, grocery stress and consumers were studied and the number of bacteria compared. Most bacteria were found on the eggs collected from the farms and least on the eggs directly collected from the homes of the consumers. Not much significant differences can be found but eggs kept under refrigeration in general seem to have fewer bacteria on the shell than eggs kept in room temperature.

Keywords: Jordan, egg shells, contamination, microorganisms, regulations

TABLE OF CONTENTS

INTRODUCTION

For a number of reasons eggs have constituted an important part of the human diet for centuries and play a significant part in religious holidays (Svenska ägg). Among these reasons are the high quality proteins of eggs, which makes them nutritious (Svenska Lantägg 2002) and the fact that the interior of the egg is protected by the shell, so that it is hard for microorganisms to contaminate the egg (The EFSA Journal 2005). Finally hens are widely used domesticated animals and relatively efficient feed converters. Holding of hens is therefore a convenient way of producing food for human consumption. The many benefits have made the use of eggs widespread around the globe (Svenska ägg).

Because of the shell the interior of the egg is protected from the surrounding environment but the egg is also a hazard due to microorganisms contaminating the eggshell when getting into contact with the manure from the hen. In this environment bacteria like *Salmonella* can grow (The EFSA Journal 2005). It is necessary to maintain good egg quality for economical viability of the worldwide egg industry (Juliet R. Roberts 2004).

Washing of eggshell is seen as a risk in most of the countries in the European Union because washing damages the outer cuticle of the shell. The cuticle offers a natural barrier to contamination and obstructs microorganisms to enter the interior of the egg. (New egg marketing measures 2004) In for example Sweden and the United States washing of the eggshell is done before the eggs are distributed to the market (Svenska Lantägg 2002, USDA 2000). This is to decrease the presence of microorganisms on the eggshell such as viruses and bacteria that can contaminate the environment around the eggs and cause food poisoning (Svenska Lantägg 2002).

Bacteria occurring in egg production systems

There are many bacteria that can contaminate the egg production system. The most common ones are *Salmonella*, *Campylobacter* and different coliforms. In the following text these bacteria and their specific characteristics are reviewed.

Salmonella is a Gram negative rod that penetrates the epithelial cell in the small intestine when entering a host. The bacteria stay there and multiply, the result is that endotoxins are released. Symptoms are diarrhea, vomiting, headache and fever and this could be life threatening if dehydration becomes severe in the body of the infected. (Garbutt, J. 1997) *Salmonella* are killed during heat treatment of food at 70 °C for at least 10 minutes (Madigan, M.T. & J.M. Martinko 2006). When homemade sauces are prepared, like mayonnaise with eggs that are not heated, *Salmonella* and other pathogens can easily cause food poisoning of the consumer. (Garbutt, J. 1997) The infectious dose of *Salmonella* varies between different serovars but can be approximately 10^5 organisms (Kothary Mahendra H. & Babu Uma S. 2001).

Campylobacter cause diseases in humans if the living bacteria are ingested. The incubation period is usually 3-5 days and the symptoms are almost the same as for

Salmonella besides abdominal pain. The infectious dose of *Campylobacter jejuni* is low and it is enough with only a few hundred cells to become sick. (Garbutt, J. 1997) According to a study by Kothary and Babu (2001) the infective dose of *C. jejuni* is 500 organisms.

When *Staphylococcus aureus* is involved in food poisoning the incubation period can be as short as 30 minutes. The symptoms are about the same as for *Salmonella* and *Campylobacter* and the mortality rate is low. As *S. aureus* grows in food an enterotoxin is produced that is the cause of food poisoning of *S. aureus*. (Garbutt, J. 1997) Approximately 100 000 bacteria per gram food are required to produce enough enterotoxin to cause disease in humans (Smittskyddsinstitutet 2006). The symptoms are mild and the duration of disease short, therefore many cases are never reported. The occurrence of *S. aureus* is not directly associated to eggs and egg products, but to handling of foods with bare hands since quite a high extent of the population of the world are carriers of the bacteria. (Garbutt, J. 1997)

Coliform bacteria are fecal bacteria that indicate some kind of fecal contamination of the food. This can easily happen when the layers get in contact with litter and if the eggs are laid directly on the floor of a floor housing system (The EFSA Journal 2005, p 7). *Escherichia coli* are a type of coliform bacteria that generally occur as a harmless member of the microflora in the gut of animals. Some strains are pathogenic and can cause food poisoning. (Garbutt, J. 1997) The infective dose of the harmless strains of *E.coli* is not interesting but pathogenic strains like 0157:H7 are extremely virulent and may have an infective dose as low as 10 organisms (Foodborne Pathogenic Microorganisms and Natural Toxins Handbook 2009). *E.coli* 0157:H7 rises an inflammation of the colon and gives abdominal pain and diarrhea, in severe cases kidneys may be damaged as well as the brain due to lack of blood platelets. (Garbutt, J. 1997)

Food poisoning symptoms include nausea and diarrhea weakens the afflicted, and for the already sick, elderly or weak it can lead to critical conditions. (Garbutt, J. 1997) Non host adapted strains of different pathogens require higher amount of bacteria to cause disease, while strains adapted to a certain host require less bacteria to cause disease (Adams and Moss 2008). In Jordan outbreaks of food poisoning is often reported in newspapers during the hot summer months but not much information about the occurrence of food borne diseases is available. (Cohen D. et al. 2010)

Aim of the study

The aim of this project was to analyze the bacterial microflora of eggshells from four different farms in the surroundings of Amman, from six minimarkets in Jordan and from three supermarkets in the capital of Jordan, Amman. Sampling has also been done from consumers where eggs are a common source of food. In this way the whole chain was studied, from stable to table, which made it possible to see if the microbiota on the egg shells is, in fact, a problem and if anything could be done to prevent it. Jordan was the place of location of this study due to that SIDA, a Swedish aid organization,

encourage and offer scholarships to students conducting small studies in developing countries. Jordan was also chosen due to that the Jordanian system differs a lot from the Swedish and European way of producing eggs. Interesting perspectives were if the hygiene of eggs in Jordan would benefit from washing or if that was not needed due to correct handling through the distribution channel by comparing it to the Swedish system.

Problem statements

The present study seeks to answer the following questions: Do the egg producers in Jordan follow the Jordanian rules and regulations for egg production regarding hygiene? How is the bacterial microflora changed from farm to grocery stores to consumers? Do any washing or cleaning of eggs occur along the production line? The first question laid the foundation of the other two and was estimated from the Jordanian standards or Chicken and chicken products – Eggs – Fresh chicken table eggs. The last question was answered by finding the answer on the second question or by talking to the managers of the farms. The study comprised two parts. First, the appliance of rules and regulations in Jordanian egg production was discussed. It was answered through the visits on farms and questions to the managers. Reviewing of the Jordanian standards regarding egg production was made and compared to the actual situation. Second the bacterial microflora was examined and compared between the different units. If a big decrease in the total bacterial number was found it may have indicated that washing really do occur along the distribution channel.

LITTERATURE REVIEW

Quality of eggs

Contaminating factors in stables

The stables are the first place where contamination of the eggshell can occur. If the stables are not clean, the egg shells are more likely to be contaminated compared to clean stables. A study from De Reu et al. (2005:1) showed that the contamination of the egg shell was not influenced by the age of the hens and they found that a higher contamination occurred in aviary systems than in cage systems. According to a study performed as early as 1970 by Quarles et al. eggs from litter-floor houses had up to 30 times more aerobic bacteria on the shell than wire-floor houses. If the excreta are moist it will make the litter moister and if eggs are laid directly at the floor or in unclean nests this can increase the microbial contamination of the shell. There is a risk that microorganisms penetrate the shell and also contaminate the interior of the egg (Smith et al. 2000). If the air is not clean and a good ventilation system is not available, dust from the straw, grain and excreta will accumulate at risk of the health of the livestock, even if bacteria is only a small part of the total dust (Pedersen S. et al. 2000). Higher bacterial contamination in the air is linked to higher bacterial counts on the egg shell (De Reu et al. 2005:2). It has been showed that the dust concentration in the air is lower in cage systems than in aviaries where it can be as much as 5 times higher than in cage systems (Ellen et al. 2000). The benefits of conventional cages offer production to a low cost and keep a good hygiene standard (Walker et al. 2001).

Packing sites

To maintain the quality of the eggs along the distribution channel, temperature and humidity should be kept at optimal levels by adequate ventilation. The design of the premises is required to facilitate sufficient cleaning, and ensure that contamination is minimized and the breeding of pests is prevented. It is also important that the buildings are spacious to be able to house all the processing equipment needed. The buildings should be clean and unneeded equipment should be discarded to minimize areas where pests can hide. The construction material of floors, walls and ceilings should be strong and smooth and in good repair to simplify sanitation. Windows and doors should be kept closed if leading to the outside, alternatively fitted with tight-fitted screens. Containers for refuse should be emptied on a daily basis and separate areas should be provided for storage of chemicals, packing materials and pesticides. It is important that areas with edible food are separated from chemicals to prevent cross contamination. To maintain the quality of eggs they must be properly handled throughout the whole distribution channel, a sanitary food environment is an integral part of the egg production industry. (USDA 2000)

Shelf life

When eggs are stored the shelf life can be prolonged if they are stored under the right conditions. The best conditions for storing eggs are in a cold, dark and odorless environment, for example a refrigerator. The eggs will stay fresh longer if stored in this

manner than in room temperature, one day in room temperature equals 4-5 days in refrigerator. If the broad end of the egg with its air bladder is held upwards the shelf life of the egg is also prolonged. When the egg gets older the chalazas slacken and the yolk floats upwards but presses against the airbladder instead of the shell. The result is a longer life span. (Svenska Lantägg 2002)

Washing

There are different opinions about whether the washing of eggs is beneficial or not. Research from Sweden has shown that washed eggs have lower microbial counts on the shell than eggs that are not washed. It was also reported that microorganisms did not penetrate the shell due to washing and did therefore not cause contamination of the interior of the egg. In Sweden consumers prefer washed eggs and great consideration is taken to the safety and rules regarding the washing procedure. (The EFSA Journal 2005) On the other hand, washing of eggs may damage the outer cuticle of the egg shell. Since this cuticle offers a barrier to contaminants, removal of it risks the quality of the egg. Also washing can cover up a low hygienic standard and poor husbandry on farms. (New egg marketing measures 2004)

If eggs are washed in cold water the content contracts and draws the water through the shell risking a contamination of the egg interior if unclean water is used. Water a little warmer than the egg is therefore to prefer. It is important that the washing procedure is performed to minimize bacterial penetration of the egg shell and that water of good quality is used. Otherwise more harm can be done than leaving the eggs dirty. It is also very important to dry the eggs completely after washing since a moist shell makes it possible for microbes to continue their growth. (USDA 2000) To reach an adequate cleaning a sanitizer-detergent must be used. If eggs are washed by water with detergent or only by water, bacteria can survive. (Board RG., Fuller R. 1994) The shell may get damaged during the washing procedure but washed eggs usually have lower microbial counts so these two factors need to be considered when deciding to wash or not. It is hard to say if the public health is affected since no epidemiological data is available but the general hygienic conditions are improved and the risk of cross-contamination in the food preparation minimized when eggs are washed. (The EFSA Journal 2005)

Food borne illnesses

A food borne illness is defined as follows by the World Health Organization (WHO): “Foodborne illnesses are defined as diseases, usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food. Every person is at risk of food borne illness.” (WHO 2007) Food borne diseases can be divided into two categories; infection and intoxication. Infection is when the food contains microorganisms that penetrate the mucosa of the intestine and cause inflammation. This can be caused by microorganisms like *Salmonella* and *Campylobacter*. Intoxication occurs when the food is contaminated of bacteria producing toxins, like the enterotoxin from *S. aureus*. One third of the reported food borne illnesses are caused by

microorganisms from the infection-group and one third from the intoxication-group. In the rest of the cases normally no cause is found. (Smittskyddsinstitutet 2006)

Jordan

The yearly number of cases of *Salmonella* in Jordan amount to 124 per 100,000 people. This is probably an underestimation and an exact number is hard to calculate as not much information is known regarding the occurrence of food borne diseases in the country. The Jordanian Ministry of Health and the Jordanian Royal Scientific Society work to map the infectious disease outbreaks and to increase the safety of the region. The name of the project is MEDICS; Middle East Consortium on Infectious Disease Surveillance and along with the Jordanian institutes works organizations from Israel and the Palestinian Authority. MEDICS first goal is to build up a surveillance system of *Salmonella* and to collect data regarding the outbreaks in the whole region. More enteric pathogens of public health importance will be mapped and put under surveillance so the outbreaks can be observed and prevented. (Cohen D. et al. 2010)

Sweden

A study conducted by the National Food Administration in Sweden found that considerably more people are food poisoned every year than what is recorded in the official statistics. The estimated number could be as many as half a million each year. From these, 50 % fall ill from food served at restaurants and 50 % from food prepared in the homes. Approximately 4 000 cases of *Salmonella* infection is reported every year in Sweden (population of 9 million), 85 % of these have been infected abroad. Food borne illnesses seems to be common but only a few cases are reported. The cause of disease is mainly a lack of knowledge or shortage in the food handling and hygiene. Sometimes the cause is too slow cooling of a food or insufficient heating; bacteria or a toxin can also get into the food from a person infected by a pathogen. In 1991 eggs from a single stock laying hens caused ten outbreaks of *Salmonella enteritidis* and many isolated diseases: one of the foods transferring the bacteria was cookies with butter cream prepared from raw eggs. (Smittskyddsinstitutet 2006)

Infective doses of bacteria

Humans at risk can suffer from infections from various microorganisms; the infective dose tells the amount of pathogenic organisms needed to cause infection. The infective dose depends on which strain it is but also on the condition in which the person afflicted by the infection finds him- or herself in. (Kothary Mahendra H., Babu Uma S. 2001)

Rules and regulations

Jordanian standards

In Jordan the standards for Chicken and chicken products – Eggs – Fresh chicken table eggs (JS 574:1988) is applied in the egg production. The standard concerns matters as definitions, quality, grading and hygienic conditions. The definition of eggs for human consumption states that the egg shell should be intact without cracks, the eggs should be examined through candling at the farm by spot-checks. The interior of the egg should

contain both egg yolk and albumen and the yolk can not be mixed with the albumen. The eggs are discharged if the albumen is dark in color or if it contains blood spots that indicate fertilization or germ cells. Grading of eggs are done by weight, eggs over 64 g are large eggs, first class between 57 and 63 g, second class between 50 and 56 g and third class below 49 g. No other grading occurs. According to the general hygienic conditions egg shells should be clean, without fecal contamination and intact. The aircell should not be more than 9.3 mm. The specific hygienic conditions state that eggs should be produced and collected in hygienic conditions from healthy birds without any zoonoses. (JS 574:1988)

Specific hygienic conditions

The production sites should be clean and a good personal hygiene should be applied among the staff. The collected eggs should be stored in a hygienic place away from sources of contamination and direct sunlight. In the grocery stores eggs free from dirt and animal feces should be offered and in the homes when eggs are broken there should be no off flavors or odors. According to the Jordanian standards eggs should be free from any pathogenic microorganisms that cause disease in human. The pathogens are not specified and this concerns the interior of the egg. (JS 574:1988)

Packing and selling

According to the Jordanian standards there are some rules to be followed when distributing eggs. The eggs should be clean and preferably washed; the washing should be done with warm water with disinfectant without smell, flavor or color, e.g. chlorine. Washed eggs should be directly dried with an appropriate method. The washing is only a recommended step that if it is done it should be done before packing on the farm. The farms producing eggs should be equipped with grading system to avoid cracked or broken eggs. Eggs should be packed in appropriate containers due to size and the containers should be clean, free from fecal contamination and off odors. The material of the containers could be either plastic or cardboard. The broad end of the egg should be held upwards when eggs are put in trays. (JS 574:1988)

Transportation and shelf life

Transportation should be done under safe and hygienic conditions to prevent cracking and air contamination according to the Jordanian standards. The vehicles used for transportation of eggs are not allowed to transport other material toxic to human. It is not mentioned under what temperature eggs should be transported, since the standards do not regulate that. If eggs are stored in the farms or at the packing sites at a temperature of 8-15°C and humidity 75-80% the shelf life should not exceed one month. If eggs instead are stored at a temperature of 4-7°C and humidity 75-80% the shelf life should not exceed two months. Nowhere in the standards is to be found how often the eggs should be collected from the farms. In grocery stores eggs should be held in hygienic places away from source of contamination and direct sunlight. The temperature should not be elevated but nothing else regarding temperature is mentioned. (JS 574:1988)

Labeling system

Each egg should be labeled with identifying color that indicates the weight of the egg. Each tray should have a label indicating the producer, trademark, date and place of packing. The labeling should be done at the farm or packing site. (JS 574:1988)

Swedish and European (EU) standards

Since Sweden is a member of the European Union the rules and regulations of the commission must be applied (EU-upplysningen 2010). The regulations are described in Council Regulation (EEC) No. 1907/90 of 26 June 1990 on certain marketing standards for eggs and in Commission Regulation (EC) No 589/2008 of 23 June 2008 laying down detailed rules for implementing Council Regulation (EC) No 1234/2007 as regards marketing standards for eggs. If applied the standards stipulated by the EU can contribute to a higher quality of eggs and egg products. Consequences of following the standards include trading benefits, which means that it is in the interest of the producers. The marketing standards also need to be reviewed regularly to guarantee that the information is correct and that the most recent research is presented. (Regulation (EEC) No. 1907/90)

The standard set up by the European Union concerns among others, matters as grading, quality, definition and labeling of eggs and egg production. The definition of eggs for human consumption states that by "'eggs' means hen eggs in shell, suitable for direct human consumption or for use in the food industries except for broken eggs, incubated eggs and cooked eggs." (Regulation (EEC) No. 1907/90, p. 2).

Grading

The regulation stipulates that the grading of eggs can only be done with instruments and methods that are suitable and have been approved for the purpose. It is also important that the quality requirements of eggs are easy for consumers to understand and that the system is "limited but adequate". Packing centers are responsible for the grading and the grading is done by quality and weight. The gradings are; class A or 'fresh eggs', class B or 'second quality or preserved eggs' and class C or 'down-graded eggs intended for the food industry'. The quality of each class is estimated according to some parameters such as appearance of the shell, consistency of the white, height of the air space, dimension and fixation of the yolk, freedom from spots and foreign bodies and development of the germ cell. (Regulation (EEC) No. 1907/90). Only eggs of class A are graded by weight and the limits are eggs over 73 g are very large eggs (XL), eggs between 63 and 73 g are large eggs (L), eggs between 53 and 63 g are medium eggs (M) and eggs with a weight below 53 g are small eggs (S) (Commission Regulation (EC) No 589/2008, *Article 4).*

Quality and washing of eggs

Eggs must meet certain standards to be graded as class A. The shell and cuticle should be normal shaped and clean without cracks. The air cell must not exceed 6 mm with a few exceptions. The yolk must be visible as a shadow when candling the eggs and the albumen should be clear and transparent. The germ should be barely visible and foreign matters or smell is not accepted. (Commission Regulation (EC) No 589/2008, Article 2)

In the European Union the washing of eggs is allowed if packing centers in the country are authorized to perform washing. The procedure and systems must conform to the national guides for egg-washing. The member states should also encourage the development of egg-washing systems and good national guides thereof. (Commission Regulation (EC) No 589/2008, Article 3). In Sweden eggs are washed at the packing centers according to national standards and the packs are labeled with the Swedish word for 'washed'. (Elvingsson P. 2010)

Shelf life and packing

The shelf life of table eggs should be stated on the pack and should not be more than 28 days after laying according to Article 13 in Commission Regulation (EC) No 589/2008. The quality of packs is also regulated by Article 17 in (EC) No 589/2008 and states that the packs used for eggs should be of good quality; shock-resistant, dry, clean and in good repair. The materials should be of a kind that protects the eggs from bad odors and reduced quality. If eggs are broken by accident in the packing procedure they must only be delivered to the processing industry, not to the food industry. (Regulation (EEC) No. 1907/90)

Labeling system

Eggs shall be presented separately under quality and weight when displayed for sale in the retail stores. When refrigeration or other methods of preservation have been used this should also be stated clearly on the package. The labeling of packs with eggs must be clear and indicate the name or business name of the producer, the address, the specific number of the plant, name, business name and the trade mark of the packing company. It must also be clearly visible on the pack what quality and weight gradings the eggs got and the type of farming they have been produced in. Eggs of class A may be labeled as 'class A' or with just the letter A together with the description of the eggs as fresh. No more identification regarding quality classes is necessary on the pack. (Regulation (EEC) No. 1907/9)

Materials and Methods

From 4 different farms 3 samples were collected from the storage room and 3 from the housing system of the hens with one exception. 4 samples were collected from 3 different grocery stores in Amman and 2 samples from 6 minimarkets each (Table 1.). One minimarket was situated at the countryside close to the city of Petra and the rest was concentrated to western Amman. 12 different consumers left one sample each, recording if the eggs were cleaned and if they normally kept the eggs in the refrigerator. In every sample 4 eggs were collected.

Table 1. Samples were collected at 4 different kinds of units and the total number of samples collected was 62, each sample consisted of 4 eggs.

Unit	Number of units visited	Number of samples	Total amount of samples
Farms	4	6 to 8	26
Minimarkets	6	2	12
Supermarkets	3	4	12
Consumers	12	1	12

The temperature in which the eggs were stored at the farms and in the grocery stores and in minimarkets was estimated. Due to the method of ISO6887-4:2003(E) each sample was placed in a stomacher bag with 100 ml Buffered Peptone water (Criterion) and the eggs were rubbed through for 1 minute. The eggs were removed from the Buffered Peptone water, their weighs were noticed and the stomacher bag with its diluents put in the Stomacher for 2 x 30 seconds.

According to the Manual of Microbiological Food Analysis for Food Laboratories from Ministry of Health in Jordan a dilution series was prepared and 0.1 ml of the diluents was plated on Tryptic Soy Agar (HiMedia) for total count and the rest of the material was incubated at 37°C for 3 hours in order to resuscitate injured bacteria. After the resuscitation the diluents were directly without any further dilution spread on Violet Red Bile Agar (OXOID), Deoxycholate citrate agar (OXOID), Campylobacter agar (OXOID) and for all samples except farms, on Baird Parker Agar (OXOID). Before plating on Baird Parker Agar a dilution series had to be made. Plates with Tryptic Soy Agar, Violet Red Bile Agar, Deoxycholate citrate agar and Baird Parker Agar were incubated for 24 h at 37°C. Plates with Campylobacter agar was incubated under microaerobic conditions for 72 h at 42°C. All samples were plated on duplicate plates.

For further identification of coliforms and *E.coli,* colonies from the Violet Red Bile Agar were transferred to test tubes with Lauryl Thryptose Broth (OXOID) and inverted Durham tubes, and incubated for 24 h at 37°C, due to the Manual of Microbiological Food Analysis for Food Laboratories. If gas was produced, 0.1 ml from each positive sample was transferred to test tubes with EC-MUG (HiMedia) with inverted Durham tubes and incubated for 24 h in water bath at 44.5°C. Samples giving positive results with gas in this test were put under UV-light to look for fluorescence.

For further identification of *Salmonella* colonies from DCA were transferred to Tripple Iron Agar-slants (HiMedia) and incubated at 37°C for 48 h. For further identification of *Campylobacter,* colonies from campylobacter agar was transferred to Tryptic Soy Agar and incubated under microaerobic conditions for 24 h at 42°C. Colonies were tested with catalase, oxidase and hippurate tests. For further identification of *S. aureus* (Manual of Microbiological Food Analysis for Food Laboratories), Gram staining was performed but also catalase- and oxidase tests.

Both confirmed and presumptive colonies were counted and reported in the results.

The following equation was used to calculate the area of the egg shell.
$S = 4.68 \times W^{2/3}$ (Wall H. et al. 2008)

The following calculation was used to express the colony forming units per cm^2 of the egg shell.

Example:

The average weight of the eggs were calculated
$W = \frac{52.1+46.1+46.2+39.8}{4} = 46.0$ g
Surface area of the egg shell was calculated by the formula above.
$S = 60.1\ cm^2$

16 CFU on plate (total count) with 10^{-4} dilution gives
$\frac{16 \times 10^4}{4} = 40\,000$ CFU/egg

$\frac{40\,000\ CFU}{60.1\ cm2} = 6.7 \times 10^2$ CFU/cm^2 of egg shell

SAS was used to calculate the statistical differences between the different units.

Results

Results from the microbial analysis of total count showed that eggs collected from farms and minimarkets are more contaminated than eggs from supermarkets and consumers (Table 2). *Salmonella* was only detected in one sample from consumers while growth on Deoxycholate citrate agar occurred on more than 50% of the samples from farms, 67% of the samples from minimarkets and 25% of the samples from supermarkets. 30% of the colonies on Deoxycholate citrate agar from farms were confirmed as *Salmonella*, while 63% from minimarkets and 67% from supermarkets were confirmed as *Salmonella*. The least amount of detected coliforms had the samples from consumers, as well as no *Campylobacter* was detected from those samples. The mean value presumptive coliforms was higher in minimarkets than in farms but the confirmed amount of *E.coli* was higher from farms than from minimarkets, 47% and 22% respectively. It is hard to say anything about the amounts of *Campylobacter* since it was only found in a few samples and only 50% of those were confirmed *Campylobacter*; most were found on eggs from minimarkets. *S. aureus* from farms was not analyzed but most *S. aureus* was found on eggs from minimarkets and least from consumers, only a few samples had no growth on Baird Parker Agar, it was mostly samples from consumers that had no growth.

Table 2. Mean value of colony forming units per cm^2 (CFU/cm^2) on eggs from farms, minimarkets, supermarkets and consumers (n. d: no colony forming units detected).

	Total count	*Salmonella*	*Coliforms*	*Campylobacter*	*S.aureus*
Farms	$11x10^5$	16	$18x10^2$	10	-
Minimarkets	$18x10^4$	$27x10^2$	$51x10^2$	22	$79x10^2$
Supermarkets	$9.7x10^2$	2.4	29	6.1	$13x10^2$
Consumers	$85x10^3$	n. d	14	n. d	$2.6x10^2$

(Mean value calculated from CFU/cm^2, samples with uncountable plates are excluded and samples with results under the detection limit are calculated as zero CFU/cm^2.)

According to the statistical analysis there were no significant differences between the different units. A statistical difference could be found regarding CFU/cm^2 on the egg shell between farms and supermarkets, where farms had a significant higher total count than supermarkets (Figure 1.).

Significant difference could be found between the amount of *Salmonella* from farms and consumers, and between minimarkets and consumers, but almost no *Salmonella* was detected in samples from consumers. Significant difference could also be found between the amount of *Salmonella* from minimarkets and supermarkets. Five of the samples from minimarkets had confirmed *Salmonella* while only two samples from supermarkets had.

Not much is to be said of the statistical analysis of coliforms and *Campylobacter* since only a few significant differences were found.

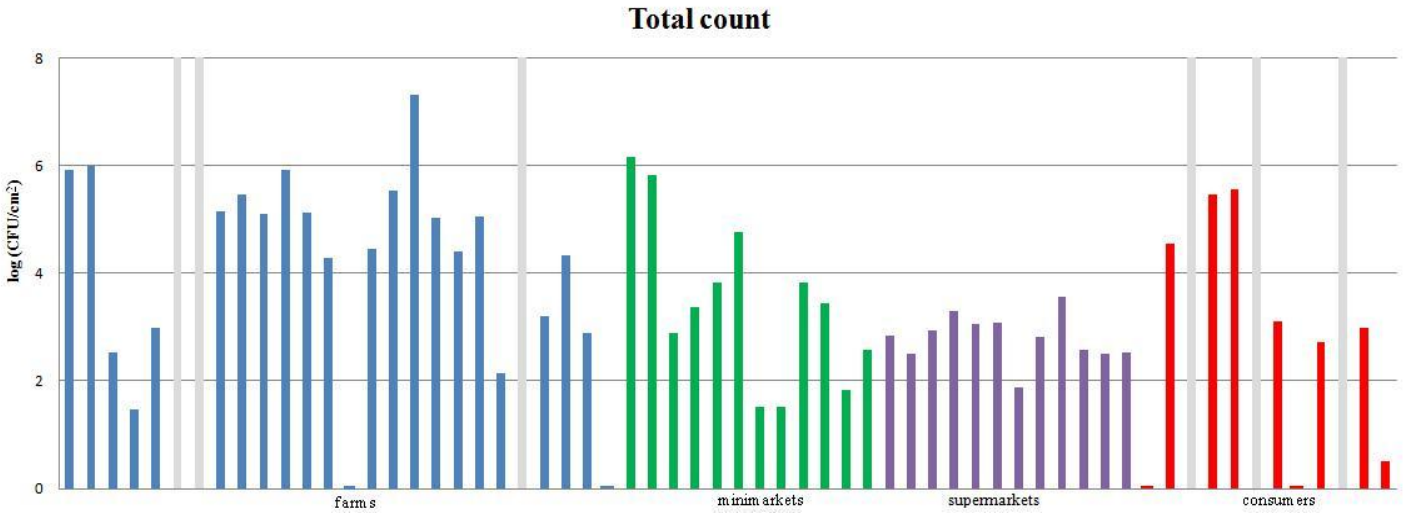

Figure 1. Diagram of total count of microorganisms on farms, minimarkets, supermarkets and consumers, grey heights emblemize uncountable samples.

Temperature

The temperature seems to have affected the total bacterial count in that way that there were more CFU/cm^2 on egg shell from consumers that store the eggs in room temperature than from the consumers that store the eggs in refrigerator. Also samples from consumers that store the eggs in room temperature had higher amount of bacteria than eggs from supermarkets, in supermarkets eggs were kept in refrigerator with one exception.

Regarding *Salmonella* minimarkets have significant higher amounts of bacteria than supermarkets as well as consumers; also more *Salmonella spp.* was confirmed from minimarkets. The same thing could be said from coliforms and *E. coli*. The values of *S. aureus* differ a lot between consumers, supermarkets and minimarkets. Minimarkets got the highest values of *S. aureus* and got significant higher or almost significant higher result of CFU/cm^2 than supermarkets and consumers.

Discussion and Conclusions

The difference between the total bacterial number on egg shells from farms and supermarkets can be explained by the time the eggs been outside the contaminating environment the stables create. The transport of eggs is not done under refrigerated conditions in Jordan but the eggs are commonly stored under refrigeration in the supermarkets, *Salmonella* does not grow when the temperature is below 6°C (Malorny B. et al. 2008). This could also be why supermarkets which store the eggs under refrigeration had lower amounts of *Salmonella* then the minimarkets that keeps the eggs in room temperature. Also consumers have significant lower amounts of *Salmonella* compared to farms, probably due to that many consumers in this study clean their eggs and store them in the refrigerator. The consumers in this study is all in some way connected to the microbiological department at University of Jordan so the results may be a bit biased since all consumers included in this study probably knows more about the risk of pathogens in food than the average citizen of Jordan and handles their food thereafter.

Since no washing or refrigeration seemed to be done at the farms included in this study the eggs reach the grocery stores in almost the same microbial conditions as they had leaving the farm. To prevent bacteria in food it is recommended to store it at a low temperature, below 4°C minimal or no growth occurs. It is also important to store the eggs for as short time as possible in an environment where cross contamination easily occurs, like the stables. Handling factors important to reduce the bacterial contamination on and in egg are frequent gathering of eggs, proper cooling and humidity conditions, proper washing and that the eggs are handled with care to avoid breakage. (Froning G.W. 1998)

The Jordanian system of egg production meets the regulations set of the Jordanian standards for Chicken and chicken products – Eggs – Fresh chicken table eggs in many ways but it is difficult to say since access to all the data was always not able to get in this study. Nothing is really said in the regulation how often the eggs should be collected from the farms but on all the visited farms the eggs was collected by a truck on a daily basis. No refrigeration occurred but that was not stated in the regulation. Many of the recommendations from the European Food Safety Authority (EFSA) and United States Department of Agriculture (USDA) listed in this text were disregarded by the egg producers. This is not that strange since those rules were not included in the Jordanian standards for Chicken and chicken products – Eggs – Fresh chicken table eggs, but anyway good to conform to.

In the Jordanian standards it is stated that eggs should be clean and preferably washed (JS 574:1988) but since visual shell contamination and bacterial contamination have very poor correlation (De Reu et al. 2005:2) it may not be enough just to estimate the eggs as clean and free from bacteria by ocular control. According to the managers on the different farms included in this study washing of eggs do not occur on their farms. In the Jordanian standards it is only a recommendation to wash eggs and not a decree (JS 574:1988); this could be why many egg producers neglect the procedure of washing.

If the eggs collected from the supermarkets are washed, they should have less microbial contamination on the egg shell than eggs directly collected from farms since washed eggs have reduced levels of contaminants on the shell (Svenska Lantägg 2002). Unfortunately it is not specified anywhere on the packs from mini- or supermarkets if the eggs are washed or not. The only conclusion drawn from this is that eggs in supermarkets may be washed but it is nowhere to be found how much washing reduces the bacterial level so there is nothing to compare the levels with.

According to a study from De Reu (2005:2) eggs from cage systems have lower contamination with total aerobic flora than eggs from floor system. Therefore may some eggs from farms in this study be cleaner than others since both farms with cage systems and farms with floor systems are included. This can also be the reason to why eggs in some supermarkets seem to be cleaner than eggs from minimarkets if minimarkets purchase their eggs from smaller farms with floor system and no washing procedure.

Food borne diseases may strike a many as 30% of the population yearly according to reports from industrialized countries. It is harder to say how many that is affected in developing countries (WHO 2007), like Jordan, where parts of the population are poor and live far from civilization and other public services. If there is a high prevalence of diarrheal diseases in a country it may indicate that the food safety is poor. Often food borne diseases occurs occasionally and is not reported and is therefore not found in the statistics, this is applied both in industrialized as well as developing countries. (WHO 2007) Reports and statistics of food borne outbreaks are hard to find in Jordan but the bigger outbreaks occurring are sometimes reported in newspaper. It is difficult to say how aware the people in Jordan are regarding food borne pathogens and food safety. The consumers included in this study are probably more aware of these aspects than the average Jordanian citizen. Often food borne diseases is spread due to lack of knowledge in hygiene and how the bacteria are spread.

Information or studies regarding the acceptable number of pathogens at the egg shell is nowhere to be found. Therefore the estimated numbers in this study can not be compared to any guidelines set up by some authority. But what is possibly to say is that the infectious dose is exceeded in many cases if comparing the infectious doses to the total CFU on eggs. Eggs had in general less *Salmonella* on the eggshell than the estimated infectious dose of 10^5 bacteria. *Campylobacter* have an estimated infectious dose of 500 organisms and if the average CFU/cm^2 is upgraded to CFU/egg more than 500 CFU can be found on eggs from farms and minimarkets. CFU is of course not the same thing as bacteria but the infectious level is reached anyway. The same thing can be said of *E. coli* and *S. aureus*.

If more samples had been collected more significant data may have been found, it would also have been interesting to collect eggs from supermarkets and farms produced by the same company and compare them. The microbiological work could be extended covering more specific methods to identify the microorganisms. If a similar study is made molecular work should be included. A further study investigating the internal

content of the eggs would be really interesting and to see how much of the external microflora that actually gets into the egg. The reason to why *Salmonella* and *Campylobacter* was enriched before plated on the medium was due to that we wanted to know the amount of *Salmonella* and *Campylobacter* at egg shells and not only if they were present there. Normally qualitative methods are used but here we tried to create quantitative methods.

This is the first project regarding the bacterial microflora on egg shell in Jordan, and it would be interesting to continue working on the same track and develop the knowledge of the hygienic status in the egg production. The Jordanian standards regarding egg production is well-intended but maybe they need to be extended and also more frequent controls should be introduced at the production sites.

Acknowledgements

This paper would not have been possible without the work of, especially, Dr Hamzah Al-Qadiri, Dr. Ayed Al Abdallat, Rana, Nadja, but also Hans Jonsson and Anders Kvarnheden are acknowledged. A special thanks to Åsa Carlsson and Sophie Jenssen Söderström, my lab partners and fellow co-workers throughout the entire project of living and working in a totally different part of the world and culture.

References

Adams Martin R, Moss Maurice O. (2008) Food Microbiology, 3rd ed, University of Surrey, Guildford, UK

Board RG, Fuller R, eds. (1994) Microbiology of the avian egg, London, p 155. Available online: www.google.com/books?hl=sv&lr=&id=5wAAJG0a5TgC&oi=fnd&pg=PA153&dq=bacterial+microflora+tableeggs&ots=f1KoOLtMLL&sig=G95t89egFwH--atzV8-bSdpndlE#v=onepage&q=&f=false

Cohen D., N. Gargouri, A. Ramlawi, Z. Abdeen, A. Belbesi, B. Al Hijawi, A. Haddadin, S. Sheikh Ali, N. AL Shuaibi, R. Bassal, R. Yishai, M. S. Green and A. Leventhal. (2010) A middle east subregional laboratory-based surveillance network on foodborne diseases established by Jordan, Israel and the Palestinian Authority, Cambridge university Press

COMMISSION REGULATION (EC) No 589/2008 of 23 June 2008 laying down detailed rules for implementing Council Regulation (EC) No 1234/2007 as regards marketing standards for eggs.

COUNCIL REGULATION (EEC) No 1907/90 of 26 June 1990 on certain marketing standards for eggs.

De Reu et al. (2005:1), Bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems for laying hens, British Poultry Science

De Reu, K., Grijspeerdt, K., Heyndrickx, M., Uyttendaele, M. & Herman, L. (2005:2) The use of total aerobic and Gram-negative flora for quality assurance in the production chain of consumption eggs. Food Control, 16: 147 - 155.

Ellen, H.H., Bottcher, R.W., von Wachebfelt, E. & Takai, H. (2000) Dust levels and control methods in poultry houses, Journal of Agricultural Safety and Health, 6:275—282

Elvingsson P. (2009) Tvättning av hönsägg, Fjäderfä nr 7-2009 Available online: fjaderfa.temp.box.se/?p=19601, 2010-05-08

EU-upplysningen, Sveriges Riksdag, Sverige i EU-domstolen [online] (2010-05-08) Available online: http://www.eu-upplysningen.se/Lagar-och-regler/Sverige-och-EU-ratten/Sverige-i-EU-domstolen/

Foodborne Pathogenic Microorganisms and Natural Toxins Handbook the "Bad Bug Book" (2009) U.S. Food and Drug Administration, Available online: http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/ucm069966.htm

Froning G.W. (1998) Egg Processing - Ideas for the Future University of Nebraska, Lincoln

Garbutt, J. (1997). Essentials of Food Microbiology. 1st ed. London*:* Arnold.

ISO 6887-4:2003(E)

Juliet R. Roberts (2004) Factors Affecting Egg Internal Quality and Egg Shell Quality in Laying Hens, J. Poult. Sci., Vol. 41: 161-177.

Manual of Microbiological Food Analysis for Food Laboratories, Ministry of Heath - Jordan

Jordanian standards for Chicken and chicken products – Eggs – Fresh chicken table eggs (JS 574:1988)

Kothary Mahendra H. Babu Uma S. (2001) Infective dose of foodborne pathogens in volunteers: A review, Journal of food safety, U.S. Food and Drug Administration Division of Virulence Assessment 200 C Street, S. W, Washington, DC 20204, ETATS-UNIS

Madigan Michael T., & John M. Martinko (2006) Brock Biology of Microorganisms, 11 ed. Pearson Prentice Hall

Malorny B., C. Löfström, M. Wagner, N. Krämer & J. Hoorfar (2008) Enumeration of *Salmonella* Bacteria in Food and Feed Samples by Real-Time PCR for Quantitative Microbial Risk Assessment, Applied and Environmental Microbiology, Mar 2008, p 1299-1304.

New egg marketing measures: EC Council Regulation adopted. Euro Surveill. 2004;8(2):pii=2359.
Available online: www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2359

Opinion of the Scientific Panel on Biological Hazards on the request from the Commission related to the Microbiological Risks on Washing of Table Eggs The EFSA Journal (2005), 269, 1-39. Available online: www.efsa.europa.eu/en/scdocs/scdoc/269.htm

Pedersen, S., Nonnenmann, M., Rautiainen, R., Demmers, T.G.M., Banbazi, T. & Lyngbye, M. (2000) Dust in pig buildings. Journal of Agricultural Safety and Health, 6:261—274.

Quarles, C.L., Gentry, R.F. & Bressler, G.O. (1970) Bacterial contamination in poultry houses and its relationship to egg hatchability. Poultry Science, 49: 60—66.

Smith, A., Rose, S.P., Wells, R.G. & Pirgozliev, V. (2000) The effect of changing the excreta moisture of caged laying hens on the excreta and the microbiol contamination of their egg shells. British Poultry Science, 41(2): 168—173.

Smittskyddsinstitutet (2006) Sjukdomsinformation om *Staphylococcus aureus* – matförgiftning. Available online: www.smittskyddsinstitutet.se/sjukdomar/staphylococcus-aureus-matforgiftning/

Smittskyddsinstitutet (2006) Sjukdomsinformation om matförgiftning. Available online: www.smittskyddsinstitutet.se/sjukdomar/matforgiftning/ 2010-05-09

Svenska lantägg 2002 2010-04-22
http://www.svenskalantagg.se/

Svenska ägg 2010-04-22
http://www.svenskaagg.se/
http://www.svenskaagg.se/?p=10935&m=3376

USDA, Egg GradingManual, 2000. Available online: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3004502

Walker, A.W., Tucker, S.A. & Elson, H.A. (2001) An economic analysis of a modified, enriched cage egg production system, British Poultry Science, 42 (Suppl.).

Wall, H., R. Tauson, and S. Sørgjerd. (2008) Bacterial Contamination of Eggshells in Furnished and Conventional Cages, Appl. Poult. Res. 17:11-16.

WHO, fact sheet N° 237, 2007, Available online: http://www.who.int/mediacentre/factsheets/fs237/en/ 2010-05-09