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Examensarbete 297
15 hp C-nivå

Swedish University of Agricultural Science 2010Department of Animal Nutrition and Management

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Abstract

This study was carried out in Jordan with the purpose to investigate the bacterial micro flora of egg shells from cage systems and from deep litter floor systems. Another aim was to get a general impression of the hygiene, at farm level, of egg production in Jordan and the factors affecting it.

Eggs from two cage systems and three floor systems were analyzed, regarding bacterial contamination of the egg shells. Egg samples were taken from the storage room, the egg cradle in the cage system and from the nest and floor in the floor system. To get a general impression of the hygiene at the farms, swab tests and air tests were performed. Swab tests were performed with a sterile cotton swab at the same units as the eggs were sampled and air tests were performed by opening petri dishes where the hens were staying. One hundred eggs from the storage room at three of the farms were also candled to examine the frequency of cracked eggs. Because the eggs were already collected from the nest/cradle, this implied that eggs with visible cracks were not included in the cracked eggs frequency.

The egg samples were rinsed in a homogenizer bag containing sterile buffered peptone water. From this solution microbial testing was performed, including spreading on agar plates in order to receive quantitative results concerning the presence of *Salmonella*, coliforms/*E. coli* and *Campylobacter*. The data were analyzed statistically.

To get an overview of the factors affecting bacterial contamination at the different farms, questions including housing system and use of antibiotics were asked. The use of antibiotics was not regulated and antibiotics was given in water or feed at most of the farms.

The cage system was significantly cleaner than the floor system regarding bacterial total count, *Salmonella* and *E. coli* on egg shells. The result regarding cracks is not reliable due to the removal if visible cracks. Regarding the hygienic aspect of egg production in Jordan much can be improved. Antibiotics and disinfectants were used without prescriptions and *Salmonella* and *Campylobacter* were found at all farms, either at the egg shell or at the egg cradle/nest. The temerarious use of antibiotics can also result in development of resistant bacteria which is a risk for the public health.

Sammanfattning

Denna studie utfördes i Jordanien med syftet att studera bakteriefloran på äggskal från bursystem respektive golvsystem. Målet var också att få ett intryck av hur hygieniska de äggproducerande gårdarnas var och vilka faktorer som påverkade bakterieförekomsten.

Ägg från två bursystem och tre golvsystem analyserades med avseende på bakterie kontaminationen av äggskalen. Ägg proverna togs från äggrännan i bursystemen och från golvsystemen togs ägg från redena och från golvet då dessa system inte hade någon ränna för äggen. Äggprover togs även från lagringsrummet. För att få ett generellt intryck av de hygieniska förhållandena togs svabb- och lufttester. Svabb testerna togs med en steril bomullspinne från samma enheter som äggproverna och lufttester utfördes genom att öppna en agarplatta där hönsen vistades. Hundra ägg lystes från tre av gårdarna för att undersökta frekvensen av ägg med knäck. Dessa ägg var inte samma som undersöktes för bakteriekontamination. Äggen lystes i lagringsrummet, vilket innebar att ägg med synlig knäck redan var bortsorterade.

För att kunna utföra en mikrobiell analys tvättades äggen i en stomacherpåse innehållandes sterilt buffrat peptonvatten. Från denna lösning utfördes sedan mikrobiella tester, bland annat genom utstrykning på agarplattor för att få kvantitativa resultat gällande förekomsten av *Salmonella, Campylobakter* och koliform/*E. coli.* Därefter analyserades resultaten statistiskt.

För att få en överblick över vilka faktorer som kan påverka bakteriekontaminationen på de olika gårdarna ställdes frågor till personalen, bland annat rörande inhysningssystemet och antibiotika användning. De flesta gårdar använde sig av antibiotika i fodret eller vattnet. Användningen var inte reglerad, det vill säga att hönshållaren kunde själv köpa antibiotika utan att en veterinär hade förordat det. Detta kan ha påverkat resultaten, men man kan inte veta hur. Jordanien har ett förbud mot försäljning av ägg om hönsen har givits antibiotika och det finns även regler för hur inhysningssystemet för hönsen ska vara utformat. Dock förekom inga kontroller för att se efter så att gårdarna följde reglerna, vilket medförde att flera av gårdarna angav att de inte följde reglerna.

Äggen från bursystemen visade sig vara signifikant mindre kontaminerade än äggen från golvsystemen gällande totala bakterieantalet, *Salmonella* och *E. coli*. Gällande hygienen finns mycket att förbättra med tanke på att antibiotika och desinfektionsmedel används utan några föreskrifter och att *Salmonella* och *Campylobakter* förekom antingen på äggskalen eller i redet/rännan på alla gårdar. Den oaktsamma användningen av antibiotika kan leda till utvecklandet av resistenta bakterier vilket är en risk för folkhälsan.

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1 Introduction

Good egg shell quality is necessary for economical viability of the worldwide egg industry (Roberts, 2004). Bad egg shell quality can result in food poisoning. The major bulk of food born outbreaks is caused by microorganisms that have the capacity to reproduce in food. Food born disease is a public health concern all over the world and can lead to chronic illness and death for the individual. For the community it is a cost for medical care, investigations and loss of productivity. The intensive farming today where bacteria and microbes can be spread through the manure is a problem. Also animal feed can be infected with pathogens and lead to cross contamination to man when eating the meat or eggs (Garbutt, 1997).

Humans, animals and plants all have an internal micro flora consisting of micro organisms. They live in symbiotic with the host body and some produce vitamins and protect us against invasion of pathogens and they are therefore essential for keeping us healthy. The pathogens are able to invade tissues or produce toxins (Garbutt, 1997).

Campylobacter and most *Salmonella* serovars are adapted animal pathogens and do not cause illness in animal but to man when transferred through e.g. eggs and meat. *Campylobacter* can contaminate eggs if manure from the hen come into contact with the eggs (Garbutt, 1997). In USA *Campylobacter jejune* is the most common cause of food borne infections (Martinko and Madigan, 2006). *Salmonella* contamination occurs from manure and bacteria can survive in dry manure for long periods. Animal feed is an important source of the bacteria as well as domestic and wild animals. *Salmonella enteritidis* is a common strain which can contaminate eggs either by contact with the manure or infect the egg as it passes down the oviduct. In man it produces a toxin that causes illness (Garbutt, 1997). An infection with *Salmonella* can cause diarrhoea, blood infection and typhoid fever. If typhoid fever is untreated, mortality in humans can reach 15 % (Martinko and Madigan, 2006). Another bacterium infecting food through contact with manure is

Escherichia coli. This bacterium is found in the normal gut flora in humans and animals. However, there are some strains such as EHEC (0157:H7) which are pathogenic for humans (Garbutt, 1997).

When leaving the cloacae most eggs are sterile. The main bacterial contamination occurs in general after eggs have been laid. Contamination occurs when the egg is in contact with nest material, trays, dust, soils and manure (Board and Tranter, 1995). Cracked eggs increase the probability of contamination inside the egg (Todd, 1996).

De Reu *et al*. (2005a) found a positive correlation between the concentration of bacteria in the air of the poultry house and the initial egg shell contamination regarding total aerobic count. This study also showed that floor eggs have a high bacterial load compared to eggs laid in nest and that the egg conveyor belt is a key point for contamination of accumulated eggs. Another study from De Reu *et al*. (2005b) reported that type of housing system can affect bacterial contamination. A higher bacterial contamination of the air from aviary systems than from cage systems and a higher total aerobic bacterial contamination on eggs from aviary system than from conventional cages were found. However, for gram-negative bacteria as *Salmonella, Campylobacter* and *E. coli* there were no higher contamination degree. The age of the hens did not affect the degree of contamination. A study by Wall *et al.* (2008) also found that the age of hens did not affect the total count or the presence of *Enterococcus*. On the other hand, a study from Kretzshmar-McCluskey *et al.* (2009) found that the microflora load on the shell increased as the age of hens increased. This is probably due to a more contaminated housing area in the end of a production period than in the beginning in some farms when the hens are young.

Singh *et al.* (2009) found that eggs from nest-boxes and floor had a higher contamination of *E. coli* and *Campylobacter* than eggs from cage system. A significant difference regarding use of nest boxes for different bird genotypes was also found. The white strains had a lower percentage of eggs laid outside the nest compared to the brown strains and hence the study suggests that there are genotype environment interactions.

Egg shell quality can be affected by bird strain as an effect of genetic selection. Brown laying strains are sometimes reported to have heavier eggs but a thinner egg shell than the white (Scott and Silversides, 2000). Increased excreta moisture, e.g. if hens are fed a too high concentration of salt, can lead to a higher egg shell contamination (Smith *et al*, 2000).

It is not possible to use a visual examination of the bacterial eggshell contamination because many studies have shown that there is no reliable correlation between visual shell contamination and bacterial contamination. Heavy soiled eggs are an exception (Board and Tranter, 1995).

In Jordan, approximately 80 percent of the laying hens are housed in deep-litter floor system equipped with nests, and remaining 20 percent are housed in conventional cages. The cage system is most common within large farms and four hens are allowed in one cage. The size of the different floor- and cage farms varies between 5000 birds up to one million at biggest farms. The average farm has 25 000 hens. Most farmers use hand collection of the eggs and commercial strains (Personal communication, Dr. Anas Al Malkawi).

There are directives regulating housing conditions of laying hens in Jordan but the Government does not check if the farms apply to these rules. Six to seven hens are allowed per square meter in the floor system and 4-5 hens per one compartment nest. Antibiotics and coccidiostats are allowed up to 16 weeks. If the layer is treated with antibiotics after 16 weeks the eggs are not allowed to be sold during the treatment (Personal communication, Dr. Hana Abdul-Hadi Zakaria).

The purpose of the present study was to investigate the bacterial micro flora of egg shells from cage systems and from floor systems and to get a general impression of the hygiene of egg production in Jordan and the factors affecting it.

2 Materials and methods

2.1 Housing, birds, feed and management

Four different farms were used (A-D). Farm A consisted of both floor (deep litter) - and cage system, farm B and C were deep litter floor systems and farm D a cage system. Questions concerning data presented in table 1 were asked to the farmers. An interpreter was used at farm B.

Tabell 1. Information about the different farms.

	A_{floor}	A_{cages}	B_{floor}	C_{floor}	D_{cages}
Age of hens at sampling	50 weeks	20 weeks	48 weeks	28 weeks	23 weeks
Breed	Hy-Line Brown	Mixed breeds/Hy-Line W36	Hy-Line W36 White	Hisex Brown and Bovans Black	Hisex White
Percentage misplaced eggs	2,2	-	1	8	-
Hens per nest or cage	10	4	5	5	5
Hens per m^2(ground floor)	10	-	5,2	7-8	-
No. of collections per day	2	2	5	2	1
Age of buildings, years	5	5	17	6	12

All farms fed their layers a mashed diet consisting of corn, soybean meal, wheat bran, calcium and a concentrate. The feed was mixed by the farms themselves and not heat treated. Feed was distributed by flat chains in all systems (except farm A_{cages} which distributed the feed manually) in the feed troughs. All farms consisted of similar buildings housing the hens with open windows which functioned as natural ventilation along the sides of the barn. The storage rooms had no cooling system, which means that the eggs were stored in room temperature.

2.1.1 Deep litter floor system

All buildings were equipped with aluminum nests situated above the ground at the long sides of the building. The nests contained varying amounts of litter. There were no egg cradles so the eggs stayed in the nests until it was collected manually. The hens had access to water bowls, perches and feed. Perches could not be used by all hens at the same time because of short length of the perches. Birds were beak trimmed at day old and then again at 12 weeks of age. All floor systems used an all in all out method with no removal of the manure before the hens were slaughtered. The manure was therefore stored in the bedding during the whole cycle. The concrete floor was covered by wood fiber or wood shavings as litter.

At farm A the buildings had a ventilation system with fans at the short ends (see fig. 1). However, the farmer mentioned that the hens suffered from heat stress during summer. The farm was situated in a desert, hence there were only fans and no cooling system, the temperature inside the building could also reach high degrees. The hens were kept in the floor system for about 50 weeks. For cleaning of the interior, water and an anti bacterial agent was used. There was also a frequent use of antibiotics in the water or feed.

Figur 1. Farm A_{floor}

At farm B (see fig. 2) the nests were removed at intervals every second week and cleaned with water and left for drying in the sun. After removal of all hens the building was cleaned with water. Antibiotics in water or feed were used when needed, which was decided by the farmer himself. A prescription for medication is not needed in Jordan and the antibiotics to apply in water or feed can be ordered from a producing factory or a veterinary (Personal communication), Dr. Hana Abdul-Hadi Zakaria).

Figur 2. Farm B

At farm C (see fig. 3) the interior was disinfected every week by spraying an anti bacterial agent dissolved in water. For the nest, granules dissolved when hens lay down on them, were used for disinfection. If the height of the deep litter manure reached over one meter during summer it was removed manually. The national directives regulating housing conditions were not applied. Antibiotic treatment was used when needed.

Figur 3. Farm C

2.1.2 Cages

All cages used were conventional cages without any equipment except from water and a feed trough with chains in front of the cages. Access to water was given with nipples at farm A and a water trough at farm D. Both farms (A and D) used water and an anti bacterial agent when cleaning. Farm A only used a few cages (see fig. 4), the rest of the cages were empty waiting for a new batch of hens to be put inside. The hens were not beak trimmed.

Figur 4. Farm A_{cages}

Farm D (see fig. 5) used beak trimmed hens. Antibiotic treatment was not used. The manure under the cages was removed daily and the building was empty for two months before new pullets were put inside. The farm claimed they applied the national rules and regulations according to housing of the hens.

Figur 5. Farm D

2.2 Sampling of data

One sample consisted of four eggs. From all farms three samples were taken from different trays in the storage room (see table 2). From the cage system, samples

were taken from the egg cradle. From floor systems, samples were taken from the nests and from eggs laid outside the nest (misplaced/floor eggs). Air tests were performed by opening two Tryptic Soy Agar petri dishes for five minutes in the housing area of farm B, C and D (see table 2). Swab tests were taken from all farms. These tests were performed by streaking a sterile cotton swab at same location as the eggs were sampled from. Thereafter the swab was placed in a tube with buffered peptone water. To examine the amount of cracked eggs, 100 eggs were candled from trays in the storage room after eye visible cracks had been removed by the operators. They were randomly sampled from all rows in different trays. From farm A, eggs collected from storage room are assumed to come primarily from floor system, hence there were only a few hens at the cage system. It was not possible to candle eggs from farm B.

Tabell 2. Sampling place and number of samplings at the different farms

	No. of air tests	No. of samples from storage room	No. of samples of misplaced eggs	No. of samples from nest/cradle	No. of eggs candled
A_{cages}	0	0	-	2	0
A_{floor}	0	3	0	3	100
B_{floor}	2	3	1	2	0
C_{floor}	2	3	1	2	100
D_{cages}	2	3	-	3	100

2.3 Bacterial identification

The egg sample was placed in a homogenizer bag containing 100 ml sterile buffered peptone water. The eggs were rinsed in the liquid for one minute. There after the liquid was put in the stomacher, a homogenizing machine transferring the bacteria to the liquid, for one minute and then enriched in 37 °C for three hours. Before enrichment 1 ml of the broth was transferred to a dilution serie and 0.1 ml of the suitable dilution was transferred to Tryptic Soy Agar (HiMedia) for total count. After enrichment 0.1 ml of the solution was spread on Violet Red Bile Agar (Oxoid), campylobacter Agar (Oxoid) and Deoxycholate citrate (Oxoid). Duplicates were made for all plates. The plates were incubated for 24-48 hours at 37 °C, except for the campylobacter agar which was incubated at 42 °C in microaerophilic conditions. The swabs were spread on the same kind of plates as the eggs.

For identification of coliform and *E. coli* Jordan's Manual of Microbiological Food Analysis 2.3 was followed with following exceptions: Spread plate technique instead of pour plate. The suspected colonies were transferred to Lauryl Tryptose Broth (Oxoid) containing Durham's tubes instead of Brilliant-Green Lactose Broth. Positive result was confirmed by development of gas after incubation at 37 °C for 24-48 hours. For detection of *E. coli* 0.1 ml from positive LTB tubes was transferred to EC-MUG Broth (HiMedia) and incubated in a water bath at 44.5 °C for 24-48 hours then point 2.5.5 and 2.5.6 was followed with the exception that no colonies were transferred to EMB. For *E. coli* identification the positive EC-MUG was checked for fluorescence under UV light. For identification of *Campylobacter*, growth on campylobacter agar during microaerophilic conditions were transferred to Tryptic Soy Agar and incubated at same conditions as before. After incubation colonies at TSA was tested for positive oxidase and katalas result. For confirmation a hippurate test was made (ISO-10272-1:2006(E)).

For *Salmonella* identification the laboratory practice was followed. Growth on selective Deoxycholate citrate agar was transferred to Tripple Iron Agar (HiMedia) slants. A yellow change in color after incubation at 37 °C for 24-48 hours confirmed presence of *Salmonella*.

All eggs were weighed and an average egg weight of each sample was calculated. The plates were counted. If two different dilutions were used, the most suitable plates containing 25-300 colony-forming units (cfu) was counted. If same dilution both plates was counted. No growth was said to be none detected. Following equation was used to express the colony forming units in cm^2/shell surface area.

S=4, 68* P exp (2/3)

S = surface in cm^2 and P = egg weight in grams (Bonnet and Mongin, 1965).

Unconfirmed bacterium was called presumptive bacteria. Presumptive bacteria are bacteria supposed to be a certain bacterium but it is not confirmed. For example, presumptive *Salmonella* grows on selective medium and look like *Salmonella* but the conformation tests were negative.

2.4 Statistical analyses

Presumptive cfu were analyzed with SAS (2009) statistical analyse system for statistical significance regarding differences between farms, floor- and cage system and sampling place. Contrast comparisons were applied when appropriate.

3 Results

3.1 Cracks and air tests

As shown in table 3 no cracks were found in eggs from cage system (D) but 2 % (C_{floor}) and 6 % (A_{floor}) cracks were found from the floor systems. This is not a reliable result which is discussed later on.

Tabell 3. Cracks, percentage

Farm	Hair crack	Star crack	Pin hole	Total
A_{floor}	2 %	4 %	0 %	6 %
C_{floor}	0 %	2 %	0 %	2 %
D_{cage}	0 %	0 %	0 %	0 %

The culture dish from farm C showed overgrowth. The air from cage system (D) had a higher bacterial count than from farm B which is a floor system (see table 4).

Tabell 4. Aerobic bacterial count from air test of hen house

Farm	Counts
B	90 cfu/plate
C	overgrowth
D	164,5 cfu/plate

3.2 Bacterial count from eggs

The two cage systems (A_{cages} and D_{cages}) had the lowest presumptive bacterial count regarding total count, *Salmonella,* coliforms*/E. Coli* and *Campylobacter* as shown in table 5. A significant difference was found between eggs from cage system and floor system regarding total count ($p<0.001$), *Salmonella* ($p<0.001$) and coliforms*/E. coli* ($p<0.001$) but not for *Campylobacter*. Eggs from the cage systems were less contaminated. This result was based on presumptive bacterial mean values from storage room and egg cradle/nest.

Tabell 5. Presumptive bacterial count in 10log cfu/cm^2 ± standard error from eggs. Bacterial count from farm B, C and D are based on a mean value on samples from storage room and egg cradle/nest. Concerning farm A, the count is based on samples from egg cradle/ nest and not from samples taken from storage room.

	A_{cages}	*[1]	A_{floor}	*	B_{floor}	*	C_{floor}	*	D_{cage}	*
Total count	2.33±0.90	-	5.96±1.10	-	4.31±0.63	-	5.29±0.63	-	2.51±0.70	-
Salmonella	0±0.26	0	1.77±0.32	57	0.69±0.19	42	0.83±0.20	30	0±0.19	0
Coliform*/E.coli*	0.68±0.42	0	3.13±0.51	48	1.07±0.30	64	0.96±0.32	48	0±0.30	0
Campylobacter	0±0.34	0	0±0.42	0	0.83±0.24	39	0±0.26	[2]	0.29±0.24	100

[1] Percentage confirmed bacterium. 0 = no confirmed bacterium.

[2] Confirmed *Campylobacter* from uncountable plate

When eggs from storage room were excluded in the analyze, eggs laid in-/outside nests in floor systems were significantly more contaminated concerning total count ($p<0.003$), *Salmonella* ($p<0.008$), and coliforms*/E. coli* ($p<0.02$) compared with cage eggs collected from the egg cradle.

Eggs laid outside the nest compared to eggs laid inside the nest in the floor system had a higher load of bacteria which is shown in table 6. However, this is not statistically analyzed.

Tabell 6. Counts of presumptive bacteria in 10log/cm^2 on eggs laid outside the nest compared to eggs laid in nest.

Place	*Total count*	*Salmonella*	*E. coli*	*Campylobacter*
$B_{Nest}1$	5,45	0,54	1,80	1,42
$B_{Nest}2$	5,09	n.d. *	n.d.	n.d.
$B_{Floor\ eggs}$	5,91	0,52	0,82	0,52
$C_{Nest}1$	4,44	n.d.	n.d.	n.d.
$C_{Nest}2$	5,53	n.d.	n.d.	n.d.
$C_{Floor\ eggs}$	7,31	uncountable	uncountable	uncountable

*n.d. = non detected bacterium

Table 7 shows that *Salmonella*, coliform*/E. Coli* and *Campylobacter* were found at all farms except from egg cradle at farm A_{cages}.

Tabell 7. Detected confirmed bacterium from swabs and eggs

Farm	Nest	Egg cradle	Storage room
A	*E. coli* *Salmonella*	[1]	*Campylobacter*
B	*E coli* *Campylobacter*	[2]	*E. coli* *Salmonella*
C	*E. coli* *Campylobacter*	[2]	*E. coli* *Campylobacter* *Salmonella*
D	[3]	*Salmonella* *Campylobacter*	*Campylobacter*

[1] None confirmed bacterium

[2] The farm has no egg cradle because it is a floor system

[3] The farm has no nest because it is a cage system

When comparing samples from storage room versus eggs collected in-/outside the nests in floor system or in the cradle in cage system, a significant difference was found regarding *Salmonella* ($p<0.02$). The eggs from storage room were more contaminated. All farms except on farm D where no *Salmonella* was detected (see table 5) from the egg samples, had a higher count of *Salmonella* on eggs from the storage room than from the cradle/nest. Farm A and C also had a significant difference regarding coliform*/E. coli* comparing samples from storage room versus egg collected at the egg cradle.

4 Discussion

4.1 Cracks

Because only eggs from storage room could be candled it is difficult to draw conclusions from the candling. There were probably a higher percentage of cracked eggs than showed in our study, because when collecting the eggs the farmers removed the cracked eggs detected at his visual checking. Farm A_{floor} had the highest percentage of cracked eggs, 6 % compared to 0% (D_{cage}) and 2% (C_{floor}) (see table 3). No cracked eggs is not a reliable result and is probably due to the sorting out of cracked eggs when collecting them from the egg cradle. According to Johansson (Kronägg AB) about one percent cracked eggs from the distributers is a good result. Factors affecting the amount of cracked eggs differ between the systems but how often the eggs are collected is important for both systems. If too many eggs are accumulated in the egg cradle, the risk for star cracks increases since star cracks are mainly caused by collision between two eggs (Lantmännen, 1980). If the nest is crowded the risk for cracked eggs will probably increase as well. The high percentage of cracked eggs at farm A_{floor} could be a result of the high-occupancy of the nest compared to the rest of the farms (see table 1). We could not see any eggs that did not roll out of the cage so this indicates that the cage floor gradient was enough. The hens at farm A are the oldest ones. This could affect the risk for cracks as the egg shell gets thinner when the hen gets older (Roberts, 2004). The age is probably the most important factor when analyzing frequency of cracks. Farm C_{floor} has young layers (28 weeks) and also a low frequency of cracks, this is comparable with farms D_{cages} where no cracks were found and the hens were 23 weeks old (see table 1 & 3). An earlier study shows that the level of cracked eggs from an aviary system can be comparable with eggs from a system with conventional cages (Abrahamsson and Tauson, 1995).

4.2 Bacterial air test

The culture dish from floor farm C showed bacterial overgrowth and this farm also had a higher total count from the egg samples than the floor farm B and the cage farm D (see table 4 and 5). Farm D had a higher mean than farm B and this is surprising because it is expected that cage system has a lower total count than the floor system (De. Reu, 2005b). It is not possible, however, to draw any conclusions from these few air tests. More tests are needed to be able to confirm that the inside air in floor system has a higher total bacterial count than air from cage system as earlier studies has shown. To be able to do the air tests and sampling of the eggs, entering inside the floor housing system was necessary but some of the hens were afraid and started flying around, especially the white hybrids. Therefore there was probably more dust in the air when performing the air tests than otherwise, but entering the housing area is a necessary also for the staff to be able to collect the eggs. High amounts of dust do not necessary mean high amount of bacteria in the air.

4.3 Bacterial egg shell analyses

Farm A_{cages} did not have any confirmed bacterium from the egg cradle and had the lowest total bacterial count (see table 5). This can be due to the fact that there were only a few cages and a smaller number of birds in the house (see fig 2) therefore the egg cradle might have been cleaner. The significantly higher *Salmonella* and coliforms*/E. coli* counts at farm A in eggs from the storage room compared with eggs sampled from the egg cradle is probably due to the fact that the eggs in the storage room, in this case, are collected from the floor system as well.

In farm C (floor) there was a significant difference between eggs in the storage room and inside the floor system concerning *Salmonella* and coliforms*/E. coli* contamination. Also here eggs from storage room were more contaminated. This may be due to further contamination during handling and/or to a further bacterial growth, since the storage room lacked a cooling system. Farm C is one of Jordan`s biggest layer farm and it is not likely that technicians who collect the eggs travel between different farms. Therefore it is most likely that the detected *Salmonella* and coliforms have its origin from farm C. In smaller farms, for example farm B, a cross contamination between different farms may be more likely.

The overall significant difference, in higher total bacterial count, *Salmonella* and coliforms/*E. Coli* for floor system compared to cage system was expected and other studies report similar results (De Reu *et al.* 2005b) (Singh *et al,* 2009).

The cleaning procedures regarding the building are important, for example *Salmonella* can survive a very long time in dry manure (Nicholson, 2004). If the building is not efficiently cleaned and disinfected before new pullets are put inside, this will lead to contamination of the new layers. Farm C had better cleaning routines than the other farms because the interior and nests were disinfected every week. Farm C also uses antibiotics but only "when needed". Considering this the farm should be very "clean". Nevertheless confirmed *Salmonella*, *E. coli* and *Campylobacter* were found and the farm had a high average of presumptive bacteria (see table 5 and 7). Even if the farms claim they only use antibiotics when needed, it could in fact, be quite a frequent use. Farm D claims they did not use antibiotics which might not be correct because there are no controls so the farms can do as they want. The fact that *Salmonella, E. coli* and *Campylobacter* are found at farm C at almost the same concentrations as at farm B despite the use of antibiotics and cleaning agents indicates that some bacteria strains could be resistant or that the cleaning procedure is not adequate enough. Resistant zoonotic bacteria strains from animals such as *Salmonella* and *Campylobacter* is a serious problem because they can be naturally transmitted to humans (SWARM 2009). Pathogenic as well as non pathogenic bacteria may develop antibiotic resistance (Van den Bogaard, 1999). Resistant non pathogenic bacteria can function as a reservoir and resistance genes can be spread to pathogenic bacteria (SWARM 2009).

At farm B the eggs were collected five times per day compared to the other farms which collected the eggs one or two times per day. If the eggs are collected more often they have less time to come in contact with manure and other layers and they should therefore be less contaminated.

Farm A_{floor} which has the highest mean of total count, *Salmonella* and *E. coli* also has the highest amount of hens per square meter and per nest (see table 1 and 5). If many layers have to share one nest it can lead to a higher risk for contamination because many hens may come in contact with the laid eggs. Jordan has laws regulating hens per square meter/nest/cage but because there are no inspections, not all farms apply the regulations.

The fact that *Salmonella, E. Coli* and *Campylobacter* were found at all farms (except for *E. coli* which was not detected at farm D)(see table 7) may depend on different factors. The mashed feed can be a source of contamination. Pelleted feed is heat treated and therefore a better choice regarding feed safety. However, in

Sweden increased problems with wet excreta and dirty eggs have been reported since the compulsory introduction of heat treatment was introduced (Wahlström *et al*, 1999). A study of McCracken *et al.* (1996) agrees with this observation. A wet excreta is a bigger problem in the floor system than in the cage system because the hens may come into contact with the manure more easily in the floor system. Wahlström *et al*, (1999) compared different strains given mashed versus crumbled diets. They found that one of the strains had significantly more dirty and misplaced eggs when fed mash diet compared to crumbled diets and this contradicts the earlier mentioned effect of mashed feed. However, birds feed the crumbled diet benefited from higher egg mass production and a lower fed conversion ratio.

Hens at farm B (floor) were older than the hens in the other farms. This could affect the bacterial contamination. This theory is supported by Kretzshmar-McCluskey *et al* (2009). On the other hand, other studies have shown that the age of the birds does not affect the bacterial contamination (De Reu, 2005b and Wall, 2008). However, the longer time the hens have spent in the building the more difficult it becomes to keep up with cleaning. In the study of Wall *et al.* (2008) only conventional cages and furnished cages were used and it is likely that the floor system is more difficult to keep clean because the manure and litter bed is only cleaned out when the hens are removed. The fact that the statistical analysis is based on presumptive counts must also be kept in mind.

4.3.1 Misplaced eggs from floor system

Limitation of nest space may increase the frequency of misplaced eggs, which may further increase the bacterial load (De Reu, 2005b). Despite that there were ten hens per nest at farm A_{floor} the percentage of floor eggs was said to be low by the farmer in the questionnaire. From floor farm C and D eggs laid outside the nest were analysed for bacterial contamination (see table 6). The misplaced eggs had a higher total count at farm C than the eggs laid in the nest. For farm B total count was only marginally higher for the misplaced eggs but the floor eggs had a higher count of *Salmonella*, *E. coli* and *Campylobacter* than sample two from the nest but not higher than sample one (see table 6), which indicates the importance of taking enough samples and representative ones.

At farm C it was only the sample from floor eggs that showed detected bacterium but the plates were uncountable (see table 6). If the plates are uncountable because of overgrowth this indicates that it is a big difference in hygiene between the floor eggs and the eggs laid in the nest. Farm C cleaned the interior of the house by spraying an anti bacterial agent every week and the nests were also

cleaned by using anti bacterial granules. This can explain why the nests seem to be much cleaner than the floor. A higher total count of aerobic bacterium and the presence of more confirmed *E. coli* and *Campylobacter* were found in misplaced eggs than from the eggs laid in the nest. Farm C had the highest percentage of floor eggs. The reason for this is unknown but Wahlström et al, 1999 reported that misplaced, cracked and dirty eggs may be affected significantly by hybrid. Especially brown hybrids can have a high amount of misplaced eggs if they do not find the nest attractive alternatively do not bother to seek for the nests at point of lay. The problem is biggest in the beginning of the laying period, when the hens are young and not used to look for the nest (Personal communication, K. Elwinger). The percentage of floor eggs were however, only an estimate reported by the farmer and hence, may not be reliable. Misplaced eggs have also a higher risk of getting broken due to pecking. Broken eggs inspire the hens to eat them. Hence, the frequency of misplaced egg can be higher than it seems (Abrahamsson and Tauson, 1998).

4.3.2 Conclusion

Eggs from a cage system seem to be less contaminated than eggs from floor systems. The egg hygiene in Jordan is most likely inadequate because antibiotics and disinfectants are used without prescription. Still *Salmonella, E. coli* and *Campylobacter* which can be transferred to man and cause illness are found in all farms. Monitoring the use of antibiotics is important for not spreading resistant bacterium which is a serious threat to public health (SWARM, 2009).

This study was a short term pilot screening study designed within the frame of available time and finance resources in order to get a general picture of the hygienic conditions on some Jordanian egg layer farms. In order to get more reliable results more duplicates and dilutions are needed. Since no farm is the other one like- e.g. age of birds - , whether it is floor or cage systems, a lot of farms would be needed to scrutinize all factors affecting the hygienic egg quality.

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