RESEARCH ARTICLE

EVOLUTION OF THE BACTERIAL CONTAMINATION RATES OF EGG INTERNAL CONTENTS DURING ITSCONSERVATION: FIRST REPORT IN ALGERIA

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ABSTRACT

This study was performed to assess the potential for exposure to some potential pathogens in table eggs from 34-week-old ISA Brown laying hens in Algeria. The study was conducted in two different laying hen houses and included a total of 504 eggs collected randomly during two distinct seasons, winter and summer, and stored at room temperature and refrigerated for 30 days. The bacteriological analysis of the different germs for the eggs collected in the winter season did not reveal any bacterial contamination for all eggs stored at room temperature and refrigerated. In the summer season, no bacteria were found in eggs kept at refrigeration temperature. Therefore, for total germs, the peak of contamination was observed for D_{14} eggs (3.09 Log CFU/g). No fecal coliform bacterial contamination was observed at D_0 and D_7. A peak fecal streptococci contamination was observed for eggs aged 21 days (2.88 Log CFU /g), and similar values were also observed for D_7, D_{14}, D_{30}, these being of the order of 2.49, 2.48 and 2.47 Log CFU /g, respectively. Only for 30-day-old eggs, coagulase-positive staphylococci were detected with a minimal contamination of about 1 Log CFU/g. These results provide useful indications on the sanitary level of the hens and the state of hygiene on the farm.

Keywords: Bacterial contamination, conservation, egg internal, laying hens

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INTRODUCTION

Meat, meat products and table eggs are foodstuffs of animal origin that can be a source of foodborne infections (Casey et al., 2012). Table eggs and egg products are consumed worldwide and constitute an inexpensive and readily available source of protein that is considered a safe food for humans (Chousalkar et al., 2021). Eggs are considered to be one of the most versatile products in the food industry due to their foaming, emulsifying, gelling and flavoring characteristics (Bhat et al., 2021).

The quality of eggs is affected in case of microbial contamination, and this can lead to the transmission of pathogens to consumers, causing food-borne infections. Therefore, the assessment of the potential risk and prevalence of microorganisms on eggs contributes to the improvement of food safety (Sodagari et al., 2020).

Microbial contamination of an egg can occur in two ways, vertical (before oviposition) and horizontal (after oviposition) (SharafEddin et al., 2019). The microbial contamination of eggshells occurs shortly after laying due to contact with the contaminated environment (Merino et al., 2019; Senbeta et al., 2015). Increasing the level of microbial load on the eggshell consequently increases the chance of penetration of microorganisms into the internal egg content (Sodagari et al., 2019; De Reu et al. 2006). In addition, the egg temperature decreases immediately after laying, creating a negative pressure inside the egg; this favors bacterial penetration (Trudeau et al., 2020).

Eggs can be contaminated by several types of pathogenic microorganisms (gram-negative and gram-positive bacteria, pathogenic fungi and mycotoxins), many of which have the ability to penetrate the eggs (SharafEddin et al., 2019, Tomczyk et al., 2018). *Staphylococcus* spp., *Salmonella* spp. and coliforms were the most germs identified (Chaemsanit et al., 2015; De Reu et al., 2008). However, different serovars of *Salmonella enterica* represent the highest threat (Paramithiotis et al., 2017).

In Algeria, to the best of our knowledge, no studies have been conducted to evaluate the internal contamination of laying hen eggs. Therefore, this is the first survey in Algeria conducted to assess the bacterial contamination of the eggs content of laying hens ISA brown and to study the effect of storage on the physical quality of eggs and the microbial load. The investigation included: total germs, total and fecal coliforms, fecal streptococci and coagulase-positive staphylococci.

MATERIAL AND METHODS

A total of 504 eggs were randomly selected during two distinct seasons at two buildings of laying hens in Algiers, North-central Algeria. 252 eggs were collected between January and March 2020, and 252 eggs were collected between July and August 2020. The sampling schedule for table eggs at the two laying hen houses is shown in Table 1.

Table 1 Sampling schedule of eggs for consumption in the two laying hen houses

Winter period		Summer period	
Batch number	Building of sampling	Batch number	Building of sampling
1	1st building	8	1st building
2	2nd building	9	2nd building
3	2nd building	10	1st building
4	1st building	11	1st building
5	1st building	12	2nd building
6	2nd building	13	1st building
7	1st building	14	2nd building

This study was conducted on the samples of table eggs from 34-week-old ISA Brown laying hens. These hens are kept in battery cages, receive 16 hours of light per day and are fed *ad libitum* with a standard layer feed. The eggs were collected as aseptically as possible using sterile gloves, then placed in sterile collection bags and transported to the laboratory in isothermal bags for analysis, on the same day.

The eggs were collected from 14 different lots (8 for the 1st building and 6 for the 2nd building) with 36 eggs per lot (Figure 1). The total number of lots studied was for both sampling periods. For each lot sampled, 4 eggs were collected on the same day of sampling for the first bacteriological analysis corresponding to D_0; the 32 remaining eggs were kept at two different temperatures: 16 were kept at room temperature in one of the ITELV premises, where the temperature and hygrometry are recorded every day. We recorded an average temperature of 17°C in winter and 28°C in summer. The 16 others were kept in a refrigerator at +4°C.

Bacteriological analysis of eggs stored at two different temperatures is performed at the 7th day ($D_{7)}$, 14th day (D_{14}), 21th day (D_{21}) and 30th day (D_{30}). The samples are transported to the laboratory in isothermal bags to limit the changes in the amount of microorganisms present.

Sample preparation for microbiological analysis of the internal of eggs

The surface of all eggs was disinfected with 70% ethanol, then the shell is opened with a sterile scalpel, the contents of the egg are then poured into a stomacher-type bag and homogenization is done in a Stomacher-type device.

Preparation of stock solutions and decimal dilutions

25 g of the whole egg homogenized with a peristaltic apparatus of the stomacher-type bag is added to 225 ml of TSE, and the solution obtained is homogenized by means of a peristaltic apparatus of the stomacher- type bag for 2 minutes; he dilutions $10^{-1,}$ 10^{-2}, 10^{-3} are then prepared according to the AFNOR standard (NF-V04-501). These dilutions will be used for the detection of total aerobic mesophilic flora, fecal coliforms, fecal streptococci and coagulase-positive staphylococci. For Salmonella, 25g of the whole egg is added to 225 ml of EPT; the resulting stock solution is homogenized by means of a peristaltic device of the stomacher type for 2 minutes.

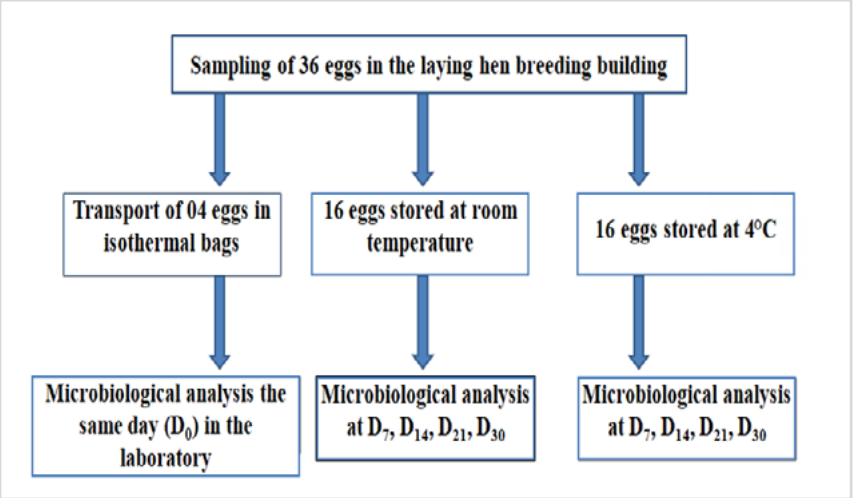

Figure 1 Diagram showing the different sampling stage

Enumeration of total germs (Standard NF V 08-51)

Enumeration was performed on PLAT COUNT AGAR (PCA) medium. Briefly, 1 ml of each decimal dilution was aseptically transferred to a separate sterile Petri dish. Then, 15 ml of the PCA agar previously dissolved and cooled to 47 °C in a water bath was placed in each Petri dish. The mixture was shaken carefully and left to solidify. Then, the second layer of 5 ml of PCA agar was added to prevent spreading of the colonies and to obtain semi-anaerobic conditions. Left it was to solidify. The plates were then placed in the incubator for 72h at 30°C. Then, the colonies were counted.

Enumeration of total coliforms and fecal coliforms (Standard NF V08-050)

The culture was carried out on VRBL. Incubation was carried out at 37°C for 24 hours for total coliforms and at 44°C for 24 hours for fecal coliforms; the red colonies were counted.

Enumeration of coagulase-positive staphylococci (Standard NF V 08 Ŕ 057 Ŕ 1)

For the isolation and enumeration of staphylococci, surface plating on Baird Parker selective medium was performed followed by an incubation of 24 to 48 h at 37°C. Characteristic colonies were counted. Microscopic observation and a search for biochemical characteristics (catalase, coagulase) were carried out.

Enumeration of fecal streptococci (Standard ISO 7899-2/ NA 766/ NF T 90-416)

Given the high number of samples taken and due to budgetary restrictions, we used the streptococcus research method adapted for water during our study. The culture was carried out on Slanetz and Bartley medium, incubation was carried out at 37°C for 24 hours, and the characteristic colonies were confirmed on Bile Esculin Agar (BEA).

Testing for Salmonella

The method for the detection of *Salmonella* is applied in accordance with the French routine standard NF V 08-52. The used medium was chromID™ *Salmonella* Agar.

Statistical analysis

All statistical analyses were performed with the R i386 3.0.2 for Windows GUI front-end statistical software. The statistical analysis of the count of different bacteria inside the eggs according to the storage period (D0, D7, D14, D21 and D30) was

studied using the Chi square and multiple range tests. For each storage period (D0, D7, D14, D21 and D30), the comparison of the average number of each bacterium between the two buildings was performed using the Student's t test. The threshold value of different tests was $P<0.05$.

RESULTS

Presentation of the results for two buildings together

The results obtained from the bacteriological analysis of eggs collected in two seasons (winter and summer) from both buildings are presented below.

In the winter season

The bacteriological analysis of the different germs for the eggs collected between January and March (winter season) did not reveal any bacterial contamination with total germs, fecal coliform, fecal streptococci, coagulase-positive staphylococci and *Salmonella* throughout the storage period for all eggs stored at room temperature and refrigerated.

However, it should be noted that when using biochemical tests to confirm some suspect *Salmonella* colonies isolated from some eggs stored at room temperature, we were able to identify three strains of *Escherichia coli* in 14-day-old eggs, one strain of *Citrobacter* in a 30-day-old egg and one strain of *Proteus* in in a 30-day-old egg.

In the summer season

No bacteria were found in the eggs kept at refrigeration temperature for the different batches in both buildings. Therefore, the results presented below will only concern eggs kept at room temperature.

Evolution of contamination of the egg internal in the summer season

The summary of the results obtained from the analysis of samples taken from a total of 252 eggs collected from two laying hen houses is shown in Table 2.

Table 2 Evolution of contamination of the egg internal in the summer season

	D0		D7		D14		D21		D30		
Eggage	**CFU/g**	**Log CFU/g**	**CFU/g**	**Log CFU/g**	**CFU/g**	**Log CFU/g**	**CFU/g**	**Log CFU/g**	**CFU/g**	**Log CFU/g**	*p*-value
TG	0.00	-	$2.7x10^1$	2.77 ± 0.16^b	$8.1x10^2$	3.09 ± 0.82^a	$2x10^3$	2.88 ± 1.03^b	$4.3x10^1$	1.88 ± 0.58^c	0.001
FC	-	-	-	-	$4.1x10^2$	3.55 ± 0.21^a	$4.8x10^2$	3.02 ± 0.56^b	$2x10^1$	1.92 ± 0.36^c	0.001
FS	0.00	-	$2.4x10^2$	2.49 ± 0.52^b	$5.3 x10^2$	2.48 ± 0.95^b	$8.6 x10^2$	2.88 ± 0.73^a	$3x10^3$	2.47 ± 0.84^b	0.001
c+Stph	-	-	-	-	-	-	-	-	0.36	1 ± 0.71	-

Chi square and multiple range tests^{a,b,c} Values that have not the same letter in the same line are significantly different at $p<0.05$.

TG: total germs; FC: fecal coliforms; FS: fecal streptococci; c+Stph: coagulase- positive staphylococci

Table 3 Results obtained from the analysis of the samples taken from eggs in the laying hen for each building and kept at room temperature

Eggs Age		**D0**		**D7**		**D14**		**D21**		**D30**	
		CFU/g	Log CFU/g	CFU/g	Log CFU/g	CFU/g	Log CFU/g	CFU/g	Log CFU/g	CFU/g	Log CFU/g
TG	**1st building**	0.00	-	0.00	-	$5.9 x10^2$	3.33 ± 0.55	$3.8 x10^2$	2.23 ± 0.84	$4.6 x 10^1$	1.89 ± 0.61
	2nd building	0.00	0.00	$81x10^1$	2.27 ± 0.16	$1.1x10^3$	2.94 ± 0.98	$4.1 x10^3$	3.54 ± 0.76	$3.9 x10^1$	1.87 ± 0.67
FC	**1st building**	0.00	-	0.00	-	$5.6 x10^2$	3.63 ± 0.21	0.00	-	0.00	-
	2nd building	0.00		0.00	-	0.00	3.38 ± 0.00	$1.1 x10^3$	3.02 ± 0.56	$3.5 x10^1$	2.01 ± 0.44
FS	**1st building**	0.00	-	$9.4 x10^1$	2.49 ± 0.52	$2.4 x10^2$	2.17 ± 0.85	$1.1 x10^3$	3.61 ± 0.15	$3.2 x 10^2$	2.50 ± 0.90
	2nd building	0.00		$4.4x10^2$	2.73 ± 0.71	$9.2 x10^2$	3.19 ± 0.88	$6.1x10^2$	2.56 ± 0.63	$2.7 x10^2$	2.42 ± 0.87
c+Stph	**1st building**	0.00	-	0.00	-	0.00	-	0.00	-	0.00	-
	2nd building	0.00		0.00	-	0.00	-	0.00	-	0.83	1.00 ± 0.00

Student's t test: Comparison between values of two buildings revealed no significant differences for each bacteria ($p>0.05$)

TG: total germs; FC:fecal coliforms; FS: fecal streptococci; c+Stph:vcoagulase-positive staphylococci

For total germs, the peak of contamination ($p<0.001$) was observed for D_{14} eggs (3.09 Log CFU/g), however, the lowest contamination ($p<0.001$) was noted for the eggs kept for 30 days (1.88 Log CFU/g). Intermediate values were found at D_7 and D_{21} with 2.77 and 2.88 Log CFU/g, respectively.

No fecal coliform bacterial contamination was observed at D_0 and D_7. On the other hand, from 14 days onwards, bacterial contamination was observed, where the values ($p<0.001$) were the highest (3.55 Log CFU/g), decreasing slightly at D_{21} (3.02 Log CFU/g), while the lowest value ($p<0.001$) was recorded for the eggs aged 30 days (1.92 Log CFU/g).

A peak fecal streptococci contamination ($p<0.001$) was observed for the eggs aged 21 days (2.88 Log CFU /g), and similar values were also observed for D_7, D_{14}, D_{30}, these being of the order of 2.49, 2.48 and 2.47 Log CFU /g, respectively.

Only for 30-day-old eggs, coagulase-positive staphylococci were detected with a minimal contamination of about 1 Log CFU/g.

Comparison of sample analyses results for each building

The summary of the results obtained from the analysis of the samples taken from eggs in the laying hen for each building and kept at room temperature is shown in Table 3.

No significant differences were found in the statistical analysis between the eggs collected from two buildings. For total germs, the eggs taken from the test house showed a maximum contamination for 14-day-old eggs (3.33 Log CFU/g). Analysis of 21 and 30-day-old eggs showed lower contamination than 14- day-old eggs (2.23 1.89 Log CFU/g), whereas 7-day-old eggs showed no contamination.

For the eggs collected from the 2nd building, the maximum contamination was observed for 21-day-old eggs (3.54 Log CFU/g). The lowest contamination value was observed for 30-day-old eggs (1.87 Log CFU/g), while intermediate values were noted for 7 and 14 day-old-eggs, which were respectively in the order of 2.27 and 2.94 Log CFU/g.

For fecal coliforms, a contamination rate for 14-day-old eggs was 3.63 Log CFU/g, while the analysis of the eggs aged 0, 7, 21 and 30 days showed no bacterial contamination. For the eggs taken from the 2nd building, the contamination rate was found for the eggs aged 14, 21 and 30 days with a peak contamination at 14 days of around 3.38 Log CFU/g and a minimum at 30 days estimated at 2.01 Log CFU/g**.**

Fecal streptococci contamination of the order of 3.02 Log CFU/g was found in 21-day-old eggs, a value close to that of 14 days. However, the peak of contamination for the eggs collected from the 2nd building was recorded for 14-day-old eggs (3.19 Log CFU/g), whereas 30-day-old eggs (2.42 Log CFU/g) were less contaminated than those from D7, D14 and D21 (2.73 and 2.56 Log CFU/g, respectively).

Bacteriological analysis for coagulase-positive staphylococci showed no contamination for the test house and for the different egg storage periods. However, the peak of contamination for the eggs collected from the 2nd building was recorded for14- day-old eggs (3.19 Log CFU/g), whereas 30-day-old eggs (2.42 Log CFU/g) were less contaminated than those at D_7, D_{14} and D_{21} (2.73 and 2.56 Log CFU/g, respectively). For the 2nd building, contamination of the order of 1 log CFU/g was found for 30-day-old eggs only.

DISCUSSION AND CONCLUSION

Microbial contamination of eggs has a serious effect on the poultry industry. It also has serious implications for public safety worldwide in terms of human disease transmission (Okorie-Kanu et al., 2016). Several factors have been implicated in egg contamination. Such is the presence of faeces, litter material, egg crates, packing and storage. Others are cloths and hands of poultry workers, dust, the environment, weather conditions, transporting and marketing (Osei Somuah et al., 2003).

In the present study, the bacteria were observed only in summer, and no bacteria were detected in winter. Indeed, it was found that the microbial contamination of eggs decreased significantly during the cold period of the year (winter). However, high temperatures (summer) could increase the degree of contamination (Mallet et al., 2006; DeReu et al., 2005a).

Egg conservation depends principally on temperature and storage time (Yuceer and Caner, 2014). The increase in temperature causes a decrease in the quality of the shell with a reduction in its thickness, which facilitates the penetration of germs inside the egg. This explains the contamination of eggs in the summer season at room temperature and its absence in the winter season (Chousalkar et al., 2021).

Our results are in accordance with the previous surveys carried out by Mallet et al. (2006) Jones et al., (2011).

At the time of laying, eggs with intact shells and from healthy hens have generally sterile content (Asamudo and Ndubuisi-Nnaji, 2017; Protais et al., 2003). The same was observed in our survey.

The risk of microbial penetration of the shell and contamination of the egg contents increases with the number of microorganisms on the eggshell (De Reu et al., 2006a). The microbial penetration in the egg is influenced by several factors, including the species of bacteria, the level of microorganisms, the method, the period and the storage conditions (Svobodová and Tůmová, 2014). Also, microbial contamination of eggs increases with storage time. De Reu et al (2007) found that the percentage of contaminated eggs increased from the first day to 21 days of storage.

In this investigation, the bacteria were detected inside the eggs from a storage period of 7 days, and the maximum number of some bacteria was observed between 14 and 21 days of storage. This is in agreement with the results of De Reu et al (2006a) and De Reu et al (2007).

The contact with any contaminated surface (hen droppings, nesting materials, dust, feed, shipping and storage containers, handlers and pets, rodents and insects) can be a source of contamination, so refrigeration of eggs is necessary to delay the growth and multiplication of microorganisms during transport and storage (Shenga et al., 2010; Olivier et al., 2009; FAO, 2003). Contamination can also occur from one egg to another (Parveen et al., 2017; Abdullah 2010; Chousalkar et al., 2010), which is also able to induce the internal egg contamination by bacterial penetration through the eggshell (Lin et al., 2021; Georgescu, et al., 2017). Therefore, storage in the refrigerator is recommended for better egg quality (Fahim et al., 2021). Eggs stored in the refrigerator recorded the lowest rate of microbial proliferation due to the bacteriostatic nature of the refrigeration temperature (Cader et al., 2014). In this study, all eggs that were kept at refrigeration temperature during the winter or summer season showed no bacterial contamination.

Therefore, it is recommended to the consumer to store eggs at less than 4°C immediately after their collection (Guedes et al., 2016). Refrigeration of

eggs allows maintaining their quality even after 20 days of storage (Serrano et al., 2016).

The investigation of germs in eggs, in this survey, shows a contamination by total germs, faecal coliforms, streptococci and staphylococci. These findings are similar to those reported by Mansour et al. (2015) and Adesiyun et al. (2005 and 2006). The total germ count revealed here was lower than the limit indicated by the E.O.S.Q.C. (2007).

The presence of pathogenic microbes in the examined samples indicates that chicken table eggs should not be consumed raw (Mansour et al., 2015).

No contamination by *Salmonella* was revealed in this survey. The absence of *Salmonella* indicates that these farms are free of salmonellosis and that the owners respect good hygiene measures. Similarly, *Salmonella* was absent in all samples investigated by Saleh et al. (2020), Mansour et al. (2015), Favier et al. (2000) and Anon (2004).

The biochemical confirmation of some suspect colonies allowed us to identify three strains of *Escherichia coli* in 14-day-old eggs, one strain of *Citrobacter* in a 30-day-old egg and one strain of *Proteus* in a 30-day-old egg. This result confirms what has been reported elsewhere (Damena et al., 2022; Saleh et al, 2020; Pereira et al, 2014).

The coliform group and streptococci are classical indicators commonly used to assess faecal contamination (Rodrigues and Cunha, 2017). Coliform bacteria classically include *Escherichia coli*, *Enterobacter* species, *Klebsiella* species and *Citrobacter* species. High coliform counts indicate unsanitary conditions or poor hygiene practices (Martin et al., 2016). Significant contamination of the shell surface can lead to contamination of the internal contents by trans-shell migration or cross-contamination during breaking (Musgrove et al., 2005). The absence of refrigerated storage may favour contamination by faecal coliforms (Damena et al., 2022). In our study, faecal coliforms were detected only in eggs stored at room temperature.

The maximum acceptable standard limit is 3 log CFU/ml of coliforms within eggs (FAO/WHO, 1983). Therefore, our results are within the standard. Similar results have been reported by El-Kholy et al. (2014) and Damena et al (2022). Other studies have reported higher values (Senbeta et al., 2015). The differences in the results of the different studies are attributed to the hygienic level of each sample.

E. coli contamination is an indicator of faecal contamination of eggs (Damena et al., 2022). In our study, only three eggs were found to be infected, which is in agreement with Chaemsanit et al. (2015) and Damena et al. (2022).

Staphylococci are part of the normal flora of humans and animals, and the presence of staphylococci in eggs reflects the history of excessive handling between laying and prolonged storage. Coagulase-positive *Staphylococcus* are considered the most important species of *Staphylococcus* spp. (Damena et al., 2022). According to the U.S. Compendium of Microbiological Criteria for Food (USDA, 2013), the number of pathogens (*Staphylococcus aureus* and other coagulase-positive staphylococci) greater than 5 log CFU/ml in foodstuffs is considered potentially hazardous. In our study, coagulase-positive staphylococci were detected in eggs from 30 days of storage at room temperature with a number of 1 log CFU/g. This meets the standards. Our findings are significantly lower compared to other studies (Damena et al., 2022, Cader et al.,2014).

This study provides useful information on seasonal changes, the effect of refrigeration and the storage time on microbial populations found in egg contents.

Further research on the effects of housing and management options, as well as on the strain of laying hen, is needed for a full understanding of the microbial implications of alternative egg production practices.

The adoption of strict hygiene measures to avoid contamination of eggs with pathogenic bacteria during the entire production, handling and storage chain

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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PROMJENE U STOPAMA KONTAMINACIJE SADRŽAJA JAJA PRI KONZERVACIJI: PRVI IZVJEŠTAJ IZ ALŽIRA

SAŽETAK

Istraživanje je provedeno s ciljem procjene mogućnosti izlaganja potencijalnim patogenima iz konzumnih jaja porijekla ISA Brown koka nesilica starih 34 sedmice u Alžiru. Istraživanje je provedeno na dvije različite farme koka nesilica, a uključilo je ukupno 504 nasumično odabrana jajeta tokom dva različita vremenska perioda, zime i ljeta, uskladištena na sobnoj temperaturi i u frižider na 30 dana. Bakteriološka analiza na različite uzročnike u jajima prikupljenim u zimskom periodu nije dokazala bakterijsku kontaminaciju jaja uskladištenih na sobnoj temperaturi i u frižideru. U ljetnom periodu bakterijski uzročnici nisu pronađeni u jajima uskladištenim u frižideru. Ako se uzmu u obzir svi uzročnici, najviša stopa kontaminacije je zabilježena u jajima od D_{14} (3.09 Log CFU/g). Nije zabilježena kontaminacija fekalnim koliformnim bakterijama tokom D_0 i D_7. Najviša stopa kontaminacije fekalnim streptokokama je zabilježena za jaja stara 21 dan (2.88 Log CFU /g), a slične vijednosti su zabilježene tokom D_7, D_{14} i D_{30}, reda veličine 2.49, 2.48 i 2.47 Log CFU/g. Samo je za jaja stara 30 dana zabilježena minimalna kontaminacija sa koagulaza pozitivnim stafilokokama od 1 Log CFU/g. Ovi rezultati su korisni indikatori sanitarne razine koka i stanja higijene na farmi.

Ključne riječi: Bakterijska kontaminacija, jaje, koke nesilice, konzervacija, sadržaj