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Isolation and Identification of Microorganisms in Eggs of a Commercial Ostrich Breeder Farm

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Abstract

Contamination of hatching eggs with pathogenic microorganisms decrease hatchability and may cause a financial loss in industrial bird production. The aim of this study was to evaluate the microbiological status of a commercial ostrich farm and its relationship to the hatchery management. Microbial sampling was done from the organs of two dead embryos, cloacae of seven newly hatched chicks and one mature female ostrich, the content of five infertile eggs, the incubation room and incubator machines, the ingredients of diet, and the shell surface of 62 eggs which were laid during a reproductive season. Various selective and differential media included MacConkey agar, blood agar, Sabouraud's dextrose agar, nutrient agar, and PPLO agar, etc. were used for isolation and identification of the microorganisms. Different types of fungi and bacterial contamination were found on the shell surface of the eggs, which Gram-positive bacilli were isolated in 74% of these eggs (46/62). From the organs of two dead embryos Gram-positive bacilli, Gram-positive cocci, *Staphylococcus epidermidis, Rhodotorula* spp., *Helminthosporium* spp., and *Aspergillus nidulans* were isolated. Most of the ingredients of the diet contained *Mucor* spp. and a wide range of bacterial species. Finally, the effect of applying the formaldehyde gas was evaluated by McNemar's test, that results showed this method was very useful for reduction of microbial load of the egg-shell in a high rate contamination. Comparison of microbial communities isolated from dead embryos and the egg-shells' surface showed that microorganisms in different parts of the farm could lead to contamination of the eggs which penetrated into the eggs. So implementing sanitation and disinfection of hatching eggs for improvement of hatchability rate is recommended.

Keywords: Bacterial and fungal contaminants; Dead embryos; Egg-shell; Incubation

Introduction

Two essential factors that demonstrate the main components of reproductive performance are fertility and hatchability [1]. Heritability of fertility and hatchability in chickens is estimated between 0.06-0.13, so, it can be concluded that the non-genetic factors have more influence on these traits [2]. The factors that influence hatchability can be quoted as the condition of the flock (age, health, nutrition, and strain), and egg conditions such as quality, sanitation, season, size, storage duration, and weight [3,4].

Attention to ostrich breeding has been increased due to the importance of ostrich products, such as meat, feathers, leather, and eggs [5]. Getting adequate profits from the breeder farms requires production high percentage of hatching egg. In the ostrich, the hatchability percentage by using the artificial incubation method is significantly lower compared with the poultry industry and reaches a maximum of about 60% on average [6]. It has been reported that the low level of hatchability of ostrich eggs in the artificial insemination is related to the presence of microbial contamination of the eggs [7]. Deeming DC, by studying on 320 ostrich eggs from nine farms reported the average microbial contamination of 22.8% of the examined eggs [8].

Meanwhile, contamination in eggs of poultry species has been reported to be 13% on average [9]. High microbial contamination in ostrich eggs with a high percentage of weight loss during incubation indicates presence of so many pores in the ostrich egg-shells [8]. After eggs laid, the egg shell surface become contaminated with the microorganisms. If the environmental conditions are suitable, they can replicate rapidly and penetrate into the egg through the shell pores. Eventually, contamination of the egg can lead to the death of the embryo [10]. Some fungi and bacteria like pseudomonads can break down the cuticle layer. Destruction of cuticle, may cause more microorganisms enter into the egg and more moisture remove from the eggs [10,11].

It has been reported by using germ-free eggs, the number of hatching eggs can be increased [12]. Therefore, for identifying sources of contamination of ostrich eggs, we tried to isolate and identify the

microbial agents in different parts of a commercial ostrich farm, and hatchery that could contaminate ostrich eggs before a flawless incubation.

Materials and Methods

Specifications of the farm

Sampling operation to investigate microbial contamination was performed in Mohases® ostrich farm in Alborz Province-Iran. The farm was located in a relatively warm and dry area, and the total number of birds was around 100 Black-Necked ostriches (*Struthio camelus australis*). In the northern hemisphere breeding season of the ostriches starts during March and ends around August/September [13], therefore, some of the produced eggs in this period were studied for microbial contamination randomly.

In this farm, the average egg production of each laying bird per month was around nine fertilized eggs. The eggs were cleaned with a brush before being placed into the incubator machine. The farm manager believed that "it was not necessary to disinfect the eggs because the eggs were laid in the sandy nests and the ostrich is a disease-resistant bird". The farm had two setters and one hatcher machines. The hatcher and setters were cleaned and disinfected before the beginning of the breeding season. During the incubation period, an antiseptic pond was being placed in front of the entrance of the incubation room.

Egg-shell sampling

To investigate the microbial contamination of ostrich eggs, 62 eggs were sampled in the sterile plastic bags with no direct contact with hands. Isolation and identification of the germs were performed with the procedure described by Deeming DC [8]. The surface of ostrich eggs was swabbed and cultured on MacConkey agar, blood agar, Sabouraud's dextrose agar, nutrient agar and PPLO agar. Then ten eggs were fumigated with formaldehyde gas for 20 minutes in a closed container and isolation and identification of the microbes were repeated again.

Environmental sampling (Incubation room: Air and Surface)

Several Petri dishes containing MacConkey agar, blood agar, Sabouraud's dextrose agar, and nutrient agar were placed in the incubation room, setter and hatcher for 15 minutes. Then, they were sealed and transferred to the lab for microbial culture. Also, the air inlet and air outlet ducts were sampled by swab and were carried over to the lab [14].

Dead embryos and infertile eggs sampling

On day 38 of incubation, when the eggs were transferred to the hatcher, the eggs were candled, and five infertile eggs and two eggs with dead embryos were selected and transmitted to the laboratory in sterile bags and were pierced with a drill. After mixing their contents, sampling was done and cultured on MacConkey agar, blood agar, Sabouraud's dextrose agar, nutrient agar, and PPLO agar. The microbial contaminations were detected in two dead embryos. The samples were taken from lung, liver, intestine, eyes, yolk sac, and heart in aseptic condition. Finally, the specimens were cultured on MacConkey agar, blood agar, Sabouraud's dextrose agar, nutrient agar, and *Salmonella-Shigella* agar [15,16].

Sampling of the cloaca

Cloacae of seven newly hatched chicks and one mature female ostrich were sampled with a wet swab randomly, and specimens were cultured in selenite broth and after 24 hours were cultured on MacConkey agar and *Salmonella-Shigella* agar [17].

Sampling of feed

To evaluate the microbial contamination of the feed, the feed ingredients, including soybean meal, alfalfa, wheat bran, bone meal, corn, barley, and mixed diet were sampled. Samples were put in sterilized polythene packets and then transferred to the laboratory. Twenty-five g of each sample was added to 225 mL of lactose broth (to make a 1:10 dilution). Then 1:100, 1:1000, and 1:10000 dilutions were prepared by adding saline. 0.5 mL of all dilutions were cultured on nutrient agar media. Also, 0.5 mL dilutions of 0.1 and 0.01 were cultured on MacConkey agar and Sabouraud's dextrose agar [18].

Table 1: Isolated bacteria and fungi from 62 contaminated ostrich eggshells.

Microorganism	Number of Isolation from the Ostrich Eggs (% of whole)
Bacteria	
Actinomycete	1 (1.6%)
Escherichia coli	1 (1.6%)
Gram-negative coccobacillus	1 (1.6%)
Gram-positive bacilli	46 (74.0%)
Klebsiella spp.	3 (4.8%)
Micrococcus spp.	2 (3.2%)
Staphylococcus epidermidis	1 (1.6%)
Fungi	
Allscheria sp.	1 (1.6%)
Alternaria spp.	6 (9.6%)
Aspergillus flavus	3 (4.8%)
Aspergillus nidulans	3 (4.8%)
Curvularia spp.	1 (1.6%)
Helminthosporium spp.	6 (9.6%)
Mucor spp.	17 (27.4%)
Penicillium spp.	4 (6.4%)
Pullularia spp.	2 (3.2%)
Rhodotorula spp.	2 (3.2%)
Scopulariopsis spp.	2 (3.2%)
Yeast	17 (27.4%)

Table 2: Isolated bacteria from the cloaca of seven newly hatched chicks and a mature female ostrich*.

Individual	MacConkey Agar	*Salmonella-Shigella* Agar
1. Chick	Neg.	Neg.
2. Chick	Neg.	Neg.
3. Chick	*E. coli*	*E. coli*
4. Chick	*E. coli*	*E. coli*
5. Chick	*E. coli*	*Klebsiella oxytoca*
6. Chick	Neg.	Neg.
7. Chick	Neg.	Neg.
*8. Mature Female Ostrich	*E. coli*	*Enterobacter agglomerans*

Neg: Negative

Sampling of water

Sampling was done to investigate the microbial contamination of the farm water. One sample was transferred to the laboratory to measure pH and water hardness. To identify the microbial contamination of farm water, MacConkey agar, blood agar, Sabouraud's dextrose agar, and nutrient agar culture media were used [19].

Microbial cultures

After sampling, they were transferred to the Laboratory of Department of Microbiology, Razi Vaccine and Serum Production Research Institute (Karaj-Iran) and microbial cultures were used to determine the type of organisms. The aerobic culture in an atmosphere of 5% carbon dioxide at 37°C overnight; and anaerobic culture incubated in a Microflow Anaerobic System cabinet providing an atmosphere of 10% carbon dioxide, 10% hydrogen and 80% nitrogen at 37°C for 48 h.

Nutrient agar is a culture medium which is used for the cultivation of microbes supporting the growth of a wide range of non-fastidious organisms that can grow variety types of bacteria and fungi. It contains many nutrients needed for bacterial growth. Blood agar is used to culture those bacteria or microbes that do not grow quickly, such as *Streptococcus pneumonia* and *Neisseria* species. It detects and differentiates hemolytic bacteria, especially *Streptococcus* species, and is a differential media for the detection of hemolysis by cytolytic toxins secreted by some bacteria, such as certain strains of *Bacillus*, *Streptococcus*, *Enterococcus*, *Staphylococcus*, and *Aerococcus*. Sabouraud's dextrose agar is appropriate for cultivating dermatophytes and other types of fungi and filamentous bacteria, such as *Nocardia*. MacConkey agar is a selective and differential culture medium for bacteria designed to selectively isolate Gram-negative and bacilli and differentiate them based on lactose fermentation. PPLO agar contains beef heart infusion and peptone to supply nutrients required for the growth of pleomorphic, filterable, fastidious microorganisms which also lack a rigid cell wall such as mycoplasmas. *Salmonella-Shigella* agar is a selective and differential medium for the isolation, cultivation and differentiation of *Salmonella* spp. and some strains of *Shigella* spp. The survey of the colonies was performed using bacteriological method [20,21].

Statistical method

The McNemar's test was used to investigate the effect of disinfection on reducing microbial contamination of ostrich eggs. The result was compared with the values of the Chi-squared table and $P<0.05$ was considered to be significant [22].

Results

None of the tested ostrich eggs or egg-shells were microbe-free. They showed a wide range of microbial contaminations (Table 1). Forty-six eggs were contaminated with Gram-positive bacilli. There were few cases of contamination with some other bacteria, such as: *Klebsiella* spp. in 3 eggs; *Micrococcus* spp. in 2 eggs; *E. coli*, *Staphylococcus epidermidis*, Gram-negative coccobacillus, and *Actinomycete* each in an egg. The recognized fungi were as follows: *Mucor* spp. and yeast each in 17 eggs; *Alternaria* spp. and *Helminthosporium* spp. each in 6 eggs; *Penicillium* spp. in 4 eggs; *Aspergillus flavus* and *Aspergillus nidulans* each in 3 eggs; *Rhodotorula* spp., *Pullularia* spp. and *Scopulariopsis* spp. each in 2 eggs; and *Allscheria* and *Curvularia* spp. each in an egg.

Sampling of the cloaca showed that none of the birds was infected with *Salmonella*, and most of the contamination was due to *Escherichia coli*. One case of *Klebsiella oxytoca* contamination was detected in one of the chicks. Swap cloaca from a mature female ostrich that indicated by number 8, presented *Enterobacter agglomerans* and *E. coli* (Table 2).

In the content of five infertile ostrich eggs, fungi were detected (i.e. *Mucor* spp. and *Helminthosporium* spp.), but not any bacteria (Table 3).

Gram-positive bacilli, *Rhodotorula* spp., *Staphylococcus epidermidis* and Gram-positive cocci in the lungs; *Helminthosporium* spp. and Gram-positive bacilli in the liver; Gram-positive bacilli, Gram-positive cocci, and *Aspergillus nidulans* in the large intestine; Gram-positive bacilli in eyes; and not any infection in the yolk sac and heart of dead embryos were detected (Table 4).

Gram-positive bacilli, *Alternaria* spp. and *Mucor* spp. from setter machines; Gram-positive bacilli and *Helminthosporium* spp. from hatcher machine; Gram-positive bacilli, *Mucor* spp., *Helminthosporium* spp. and *Aspergillus nidulans* from setter room; Gram-positive bacilli, *Mucor* spp. and *Alternaria* spp. from hatcher room; Gram-positive bacilli, *Mucor* spp. and *Helminthosporium* spp. from air inlet duct; and Gram-positive bacilli, *Alternaria* spp. and *Penicillium* spp. from air outlet duct were isolated (Table 5).

Bacillus spp., *Klebsiella* spp., *E. coli*, and *Mucor* spp. from alfalfa were isolated. Gram-positive bacilli and yeast in soybean meal; Gram-negative *Coccobacillius* and *Mucor* spp. in wheat bran; Gram-positive bacilli, *Mucor* spp., *Helminthosporium* spp., *Aspergillus nidulans*, and *Scopulariopsis* spp. in bone meal; *Mucor* spp. and Gram-positive bacilli in corn; *Mucor spp.*, *Rhodotorula* spp., and Gram-negative Coccobacillius in barley; and *Mucor* spp., *Bacillus* spp., and *Klebsiella* spp. in mixed diet were found (Table 6).

Drinking water of the farm was free of microbial contamination. The degree of water hardness, alkalinity, and pH was 217 ppm, 113 ppm, and 8, respectively.

Disinfection with formaldehyde gas showed a significant impact on the reduction of the bacterial and fungal load in nutrient agar and Sabouraud's agar ($P<0.05$). These results indicate that disinfection with formaldehyde gas is useful when the rate of microbial contamination is high (Table 7).

Discussion and Conclusion

Microbial contamination can transmit vertically or horizontally from breeders to eggs. In the vertical route, bacteria may be colonized in ovaries or oviducts and the eggs become infected. Although in our study, the type of isolated microbes in the mature female's cloaca and content of infertile eggs was not similar, but, the risk of transmission of microbial contamination from the reproductive system of female birds to eggs is very high. For example, egg-shells can be contaminated with *Salmonella* trough the oviduct or faecal carriage [23]. In the horizontal rout, the microbes can contaminate the egg-shell through the environment, such as faecal material or dust and infect the membranes and yolk [9]. Due to the formation of spores and drought resistance, Gram-positive rods can dominate the egg-shell microflora. It has been reported that some opportunistic germs, such as some fungi, Gram-positive cocci, Gram-negative enteric, and Gram-negative fermenters can infect egg contents [24,25]. If the environmental conditions are suitable for microbial growth, microbes can multiply rapidly in freshly laid eggs and penetrate the egg-shell through the pores. Eventually, this situation can lead to embryos' death and a reduction in the hatching rate [10].

In a scientific report study, *Bacillus* spp., *Staphylococcus* spp., *Klebsiella* spp., *Escherichia coli*, and *Proteus* spp. were isolated from

Klebsiella spp., *Escherichia coli*, and *Proteus* spp. were isolated from twelve dead embryos of the ostrich [26]. *Staphylococcus* spp. also was found in dead embryos in the present study, and *Bacillus* spp., *Klebsiella* spp., and *Escherichia coli* existed on the egg-shells of our research. All microbes that exist in table 4 have also been reported in table 1. So it can be concluded that the contamination through the egg-shell has penetrated into the eggs. Gram-positive bacilli that have been detected in the lung, liver, intestine and the eyes of the dead embryos also have been isolated in almost all areas of the farm, such as feed ingredients (Table 5), incubation room, setter and hatcher machines and air inlet and air outlet ducts of incubation rooms (Table 6). The presence of contaminants in infertile eggs can be a reason for the high rate of early embryonic mortality in the ostrich eggs [7].

Table 3: Isolated microbes from the content of five infertile ostrich eggs.

No. of Ostrich Eggs/Culture media	MacConkey agar	Blood agar	Sabouraud's dextrose agar	Nutrient agar	PPLO agar
1	Neg.	Neg.	Neg.	Neg.	Neg.
2	Neg.	Neg.	*Helminthosporium* spp.	Neg.	Neg.
3	Neg.	Neg.	*Mucor* spp.	Neg.	Neg.
4	Neg.	Neg.	Neg.	Neg.	Neg.
5	Neg.	Neg.	*Helminthosporium* spp.	Neg.	Neg.

Neg: Negative

Table 4: Isolated microbes from different organs of the two dead embryos.

Organ/Culture media	MacConkey agar	Blood agar	Sabouraud's dextrose agar	Nutrient agar	PPLO Agar	Salmonella-Shigella agar
Lung	Neg.	Gram-positive bacilli	*Rhodotorula* spp.	*Staphylococcus epidermidis*, Gram-positive bacilli, Gram-positive cocci	Neg.	Neg.
Liver	Neg.	Neg.	*Helminthosporium* spp.	Gram-positive bacilli	Neg.	Neg.
Large Intestine	Neg.	Gram-positive bacilli	*Aspergillus nidulans*	Gram-positive bacilli, Gram-positive cocci	Neg.	Neg.
Eyes	Neg.	Neg.	Neg.	Gram-positive bacilli	Neg.	Neg.
Yolk Sac	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Heart	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

Neg: Negative

Table 5: Isolated microbes from different parts of the incubation room.

Sampling location /Culture media	MacConkey agar	Blood agar	Sabouraud's dextrose agar	Nutrient agar
Setter machine (swab)	Neg.	Neg.	*Mucor* spp.	Neg.
Hatcher machine (swab)	Neg.	Gram-positive bacilli	*Helminthosporium* spp.	Gram-positive bacilli
Setter machine (Petri dish)	Neg.	Gram-positive bacilli	*Alternaria* spp.	Gram-positive bacilli
Hatcher machine (Petri dish)	Neg.	Neg.	*Helminthosporium* spp.	Gram-positive bacilli
Setter room	Neg.	Gram-positive bacilli	*Mucor* spp., *Helminthosporium* spp., *Aspergillus nidulans*	Gram-positive bacilli
Hatcher room	Neg.	Gram-positive bacilli	*Mucor* spp., *Alternaria* spp.	Gram-positive bacilli
Air inlet duct	Neg.	Gram-positive bacilli	*Mucor* spp., *Helminthosporium* spp.	Gram-positive bacilli
Air outlet duct	Neg.	Gram-positive bacilli	*Alternaria* spp., *Penicillium* spp.	Gram-positive bacilli

Neg: Negative

Table 6: Isolated germs from feed ingredients.

Feed Items/Culture media	Bacteria	Fungi
Soybean meal	Gram-positive bacilli	Yeast
Alfalfa	*Bacillus* spp., *E. coli, Klebsiella* spp.	*Mucor* spp.
Wheat bran	Gram-negative coccobacillus	*Mucor* spp.
Bone meal	Gram-positive bacilli	*Mucor* spp., *Helminthosporium* spp., *Aspergillus nidulans*, *Scopulariopsis* spp.
Corn	Gram-positive bacilli	*Mucor* spp.
Barley	Gram-negative coccobacillus	*Mucor* spp., *Rhodotorula* spp.
Mixed diet	Bacillus, *Klebsiella* spp.	*Mucor* spp.

Table 7: Microbial culture results of 10 ostrich eggs before and after disinfection with formaldehyde gas.

	Culture media				
Microbial status of egg-shells before/ after disinfection*	**MacConkey agar**	**Blood agar**	**Sabouraud's agar**	**Nutrient agar**	**PPLO agar**
Negative/negative	6	1	2	0	7
Negative/positive	0	1	0	0	0
Positive/negative	4	7	6	8	3
Positive/positive	0	1	2	2	0
χ^2 **	2.25	3.12	4.16	6.12	1.33
P-value	0.13	0.07	0.04	0.01	0.24

*Positive: Growth; Negative: No Growth

**McNemar chi-squared statistic with Yates correction of 1.0; χ^2 value exceeds the table value of 3.841 (at 1 degree of freedom and an alpha level of 0.05)

twelve dead embryos of the ostrich [26]. *Staphylococcus* spp. also was found in dead embryos in the present study, and *Bacillus* spp., *Klebsiella* spp., and *Escherichia coli* existed on the egg-shells of our research. All microbes that exist in table 4 have also been reported in table 1. So it can be concluded that the contamination through the egg-shell has penetrated into the eggs. Gram-positive bacilli that have been detected in the lung, liver, intestine and the eyes of the dead embryos also have been isolated in almost all areas of the farm, such as feed ingredients (Table 5), incubation room, setter and hatcher machines and air inlet and air outlet ducts of incubation rooms (Table 6). The presence of contaminants in infertile eggs can be a reason for the high rate of early embryonic mortality in the ostrich eggs [8].

Deeming DC [9] examined microbial contaminations in ostrich eggs. He reported six cases of fungal contamination in ostrich egg-shells that four of them were found in the present study. Previous studies indicated that the role of bacteria is more prominent in the contamination of eggs than fungi. However, it is stated that the addling of eggs strongly related to the attendance of fungi on egg-shells [28]. Fungal spores are almost present everywhere. Fungi can cause a variety of infectious diseases and intoxications due to their high numbers and prevalence and rapid spore release. Eggs have limited chemical defense against fungi (e.g. no chitinases). Fungi can break down the shell cuticle. As a result, the egg-shell pores would be accessed for bacteria and they can quickly enter into the eggs [11,29].

Some papers reported that ostrich eggs do not have a cuticle layer (Deeming, 1995), while some researchers have reported its existence [30]. Therefore, because of this contradiction, it is necessary to minimize the probability of contamination of eggs with preventive methods. Studies conducted under laboratory conditions have shown that the presence of water is necessary for penetration of microbes into the egg-shells. Because the moisture leads to the proliferation of bacteria on the egg-shells and by penetrating the shell pores, it acts as a medium for the transmission of microbes [11,29]. Thus the nest of ostriches should be well dry, be covered with a grainy litter, and dust should be cleaned regularly.

In the farm under our study, the eggs were collected after being laid and the environment of the farm was usually kept clean. Unfortunately, the disinfection of in incubated eggs was not performed and the disinfection of incubation rooms and incubator machines were only administered at the beginning of the breeding season, and no vaccination was done.

Due to increasing the diffusion resistance in the pores of egg-shells and reducing gaseous exchange, the washing of eggs is not recommended. Also, if the temperature of the disinfectant solution is lower than the temperature of the egg, the volume of the egg content reduced. This causes a negative pressure and a vacuum that moves bacteria to the internal contents of the egg through the pores of the shell, and the embryo becomes infected [7,30]. Since the usefulness of using a mixture of permanganate-potassium formalin to disinfection of ostrich eggs is questionable due to its strong oxidizing properties [7], our results showed this method of disinfection was beneficial for the reduction of microbial load of the egg-shell in a high rate contamination. But in a low rate of contamination it was not very effective and can be ignored with some measures and practices, such as implementing sanitation and hygiene e.g. quick collection of eggs [31], the maintenance of nest hygiene [32], carefully gathering and wiping the eggs with a dry cloth, and holding the eggs with sterile toweling [33].

Average hatchability of incubated eggs in the commercial ostrich farms have ranged from 37.5% to 61.8% [6]. In commercial farms, even a small increase in hatchability leads to more economic benefit, so everything should be done to its improvement. Therefore, due to the role of microbial contamination in the reduction of hatchability and the uncertainty about the presence of cuticle in ostrich eggs, rigid standards of sanitation must be maintained in everywhere in the ostrich farm.

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References

1. Abd El-Hack ME, Hurtado CB, Toro DM, Alagawany M, Abdelfattah EM, et al. (2019) Fertility and hatchability in duck eggs. World's Poult Sci J 75: 599-608.
2. King'Ori AM (2011) Review of the factors that influence egg fertility and hatchability in poultry. Int J Poult Sci 10: 483-492.
3. Elibol O, Peak SD, Brake J (2002) Effect of flock age, length of egg storage, and frequency of turning during storage on hatchability of broiler hatching eggs. Poult Sci 81: 945-950.
4. Yassin H, Velthuis AG, Boerjan M, van Riel J, Huirne RB (2008) Field study on broiler eggs hatchability. Poult Sci 87: 2408-2417.
5. Khosravi AR, Shokri H, Ziglari T, Naeini AR, Mousavi Z, et al. (2008) Outbreak of severe disseminated *Aspergillosis* in a flock of ostrich (*Struthio camelus*). Mycoses 51: 557-559.

6. Bronneberg RG, Taverne MA (2003) Ultrasonography of the female reproductive organs in farmed ostriches (*Struthio camelus* spp.). Theriogenology 60: 617-633.

7. Cooper RG (2001) Handling, incubation, and hatchability of ostrich (*Struthio camelus* var. domesticus) eggs: a review. J Appl Poult Res 10: 262-273.

8. Deeming DC (1995) Factors affecting hatchability during commercial incubation of ostrich (*Struthio camelus*) eggs. British Poult Sci 36: 51-65.

9. Bruce J, Drysdale EM (1994) Trans-shell transmission. In: Board RG, Fuller R (eds) Microbiology of the Avian Egg. Chapman and Hall, London 63-91.

10. Cook MI, Beissinger SR, Toranzos GA, Arendt WJ (2005) Incubation reduces microbial growth on eggshells and the opportunity for trans-shell infection. Ecol Lett 8: 532-537.

11. Board RG, Loseby S, Miles VR (1979) A note on microbial growth on hen egg shells. British Poult Sci 20: 413-420.

12. Dzoma BM (2010) Some Factors Affecting Fertility and Hatchability in the Farmed Ostrich: A Review. J Anim Vet Adv 9: 229-239.

13. Leuthold W (1977) Notes on the breeding biology of the ostrich *Struthio camelus* in Tsavo East National Park, Kenya. Ibis 119: 541-544.

14. Berrang ME, Cox NA, Bailey JS (1995) Measuring Air-Borne Microbial Contamination of Broiler Hatching Cabinets. J Appl Poult Res 4: 83-87.

15. Khan MA, Ashfaque M, Hussain I (1999) Bacteriology of Dead-in-Shell Broiler Embryos and Antibiotic Sensitivity of the Isolates. Pak J Biol Sci 2: 442-444.

16. Rezaei Far A, Peighambari SM, Sadrzadeh A, Askari Badouei M (2013) Bacterial contamination of dead-in-shell embryos in ostrich hatcheries and antimicrobial resistance patterns of isolated *Escherichia coli*. Iran J Vet Med 7: 169-175.

17. Afriyani A, Darmawi D, Fakhrurrazi F, Manaf ZH, Abrar M, et al. (2016) Isolation of *Salmonella* sp. in feces of broiler chicks at Ulee Kareng Market Banda Aceh. J Med Vet 10: 74-76.

18. Danbappa AAR, Alhassan KA, Shah MM (2018) Isolation and identification of microbial contaminants associated with commercial poultry feeds. Journal of Applied and Advanced Research 3: 142-147.

19. Walker JT, Bradshaw DJ, Bennett AM, Fulford MR, Martin MV, et al. (2000) Microbial biofilm formation and contamination of dental-unit water systems in general dental practice. Appl Environ Microbiol 66: 3363-3367.

20. Swayne DE, American Association of Avian Pathologists (1998) A laboratory manual for the isolation and identification of avian pathogens. 4th Edition, The American Association of Avian Pathologists, Pennsylvania, USA 4-16.

21. Quinn PJ, Markey BK, Carter ME, Donnelly WJ, Leonard FC (2001) Veterinary Microbiology and Microbial Disease. 1st Edition, Blackwell Science, Oxford 43-122.

22. Fagerland MW, Lydersen S, Laake P (2013) The McNemar test for binary matched-pairs data: mid-p and asymptotic are better than exact conditional. BMC Med Res Methodol 13: 91.

23. Humphrey TJ (1994) Contamination of eggs with potential human pathogens. In: Board RG, Fullar R (eds) Microbiology of the avian egg. Chapman and Hall, London 93-116.

24. Kozlowski S, Malysko E, Pinowski J, Kruszewicz A (1989) The effect of microorganisms on the mortality of house sparrow (*Passer domesticus*) and tree sparrow (*Passer montanus*) embryos. Intecol Slupsk Poland 1: 121-128.

25. Houston CS, Saunders JR, Crawford RD (1997) Aerobic bacterial flora of addled raptor eggs in Saskatchewan. J Wild Dis 33: 328-331.

26. Jahantigh M (2010) Bacteriological study of dead-in-shell embryos of ostrich, scientific report. J Vet Res 11: 88-90.

27. Cook MI, Beissinger SR, Toranzos GA, Rodriguez RA, Arendt WJ (2003) Trans-shell infection by pathogenic micro-organisms reduces the shelf life of non-incubated bird's eggs: a constraint on the onset of incubation? Proc Biol Sci 270: 2233-2240.

28. Board RG, Halls NA (1973) The cuticle: a barrier to liquid and particle penetration of the shell of the hen's egg. Br Poult Sci 14: 69-97.

29. Richards PDG, Botha A, Richards PA (2002) Morphological and histochemical observations of the organic components of ostrich eggshell. J S Afr Vet Assoc 73: 13-22.

30. Tøien Ø, Paganelli CV, Rahn H, Johnson RR (1988) Diffusive resistance of avian eggshell pores. Respir Physiol 74: 345-354.

31. Button C, Moon D, Turner D (1994) Increasing the hatchability of ostrich eggs. Aust Ostrich Assoc J 27: 18-23.

32. Deeming DC (1996) Production, fertility and hatchability of ostrich (*Struthio camelus*) eggs on a farm in the United Kingdom. Anim Sci 63: 329-336.

33. Cooper RG (2000) Treat ostrich eggs with care. World Poult 16: 33.