

Prevalence of Escherichia Coli and Salmonella Species in Ostrich Farms in Egypt

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Abstract: This study aimed to monitoring the microbial status of ostrich farms through isolation of bacteria causing disease especially family Enterobacteriaceae from chicks and adult yards. And identification of isolated microorganism by serological tests and PCR. A total of 273 samples were collected from adult and chicks yards including: freshly feces, soil, feed and water; our results revealed that, the overall prevalence of *E. coli* in all examined samples from chick's yards and adult yards was 58.4% (59/101) and 62.7% (108/172) respectively and the overall prevalence of salmonella spp. in all examined samples from chick's yards and adult yards was 20.8% (21/101) and 24.4% (42/172) respectively. The obtained results indicated that, ostrich droppings is one of the most important sources of *E. coli* and *Salmonella* in ostrich farms, serotyping of isolated *E. coli* and *Salmonella* serovars strains and detection of virulence genes by PCR was performed in our study.

Keywords: Ostrich-chicks-*E. coli*-*Salmonella*-serotyping-PCR.

I. Introduction

Ostrich farming has been rapidly expanding in Egypt to produce usable products such as meat, hides, feathers, and eggs. Ostrich (*Struthio camelus* var. *domesticus*) raising needs experience and information from farmers and the successful ostrich farming is largely dependent on the ability of farmers to rear sufficient numbers of viable and healthy chicks. [1]

Ostrich environment and its microbial load play a significant role in influencing the growth performance of ostrich and thus affect the quality of ostrich product. Ostrich meat and other products can be sources for human infections and may get contaminated through handling, processing, cooking, packaging and storage. Such contamination with pathogenic microorganisms not only renders ostrich products unfit for human consumption but also increases human risk [2]. Meat quality is dependent on the entire meat production chain from the farm where animals are conceived to the consumer [3].

In Egypt, industrial cycle begins with hatchery which collects fertile eggs from the breeder farms incubates them and finally sells the newly hatched chicks to the commercial ostrich farms. Good hygiene practices should apply in all ostrich production sectors especially in adult yards as it is very important to reduce the contamination with microorganisms in other parts of farm (hatcheries, eggs and chicks yards). The pathogenic microorganisms which can be isolated from hatching eggs can be easily distributed to other places through air movements during hatching and as a result all other chicks in the hatchery can be contaminated [4] producing diseased chicks which continue in the production cycle.

Housing design also contributes to the level of microbes in ostrich bodies as ostriches penned on cement or tiles are restless and defecate readily when compared to those penned on sand. Cement or tiled flooring becomes wet and soiled and when ostriches lie down, expensive body feathers are soiled with faeces and urine. On the other hand, ostriches penned on sand are less restless and defecate less. Another advantage of sand is that the urine drains away in the sand, keeping the surface dry, so that when ostriches lie down their feathers are less soiled [5]. The environment of a farm with heavy soil and poor drainage often results in animals arriving at the abattoir with muddy feet and abdomens. Dirty skins provide major sources of microbial contamination for the carcass. [6]

Ostrich are susceptible to a number of infectious agents which are common to other avian species. They have no infectious or contagious species specific diseases [7]. The bacterial pathogens most frequently involved in infectious enteritis of ostriches are: *Escherichia coli* (*E. coli*), *Salmonella* spp. [8]. *E. coli* contaminate environmental sources (vegetation, soil and water) contribute to exposure, soon after birth [9]. Some *E. coli* strains are pathogenic and have been associated with specific diseases in humans and animals: gastroenteritis, urogenital disease, septicemia, and pleural infections [10]. *Salmonella* was isolated from ratites birds 5 days to 4 years of age [11]. The affected birds were from flocks that had fence-to-fence contact with other animal species, such as pigs, goats, free-roaming guinea fowl or domestic turkeys. Different *Salmonella* serotypes cause enteritis in ostriches especially chicks. The clinical sign of diarrhea is often observed and in some instances sudden death may occur. Other cases may display nonspecific signs of anorexia and depression [12].

This study aimed to Monitoring the microbial status of ostrich farms through: Isolation of bacteria causing disease especially familyEnterobacteriaceae from chicks and adult yards environment. and Identification of isolated microorganism by serological tests and PCR

II. Materials And Methods

Ostrich farms:

The present study was carried out in six ostrich farms located at (Giza, Bani-swef, El-marg, Ismailia, El Obor and 10th of Ramadan) at the period between April 2015 up to December 2015. Three farms contain Hatchery. Each farm was divided into chick yards (up to 1 months age) and adult productive yards (males and females different in age). The soil of adult yards differ from dust to sand while in chicks housed on cement floor covered by thin layer of lime. Most farms are breeding other animal species with ostrich separated by fence.

Sampling:

A total of 273 samples were collected from Adult and chicks yards including: Freshly feces, soil, feed and water, Samples are transported to bacteriological laboratory as soon as possible under complete sterile conditions (in an ice box).

Isolation of E. coli and Salmonella from different samples:

Bacterial isolation: was carried according to Cruick Shank et al.[13] .1 gm of feces, soil, feed and 1 ml of water samples were aseptically transferred to 9 ml buffer peptone water and other , Then transfer 0.1 ml from pre-enrichment tubes to tubes containing Rappaport-Vassiliadis Soya broth (RVS). The inoculated media was incubated at $42 \pm 0.5 \text{ }^{\circ}\text{C}$ for 18-24 hours. A loopfull from buffer peptone water was streaked onto MacConkey's agar, Eosine methylene blue agar (EMB). The inoculated plates were incubated at 37°C for 24 hours. Another loopfull was taken from RVS broth and streaked onto xylose lysine deoxycholate (X.L.D) plates were incubated at 37°C for 24 hours aerobically.

Identification of isolated salmonella and E coli spp.

biochemical identification :

Purified isolates were examined by different biochemical reactions including oxidase, urea hydrolysis, H₂S production on TSI, lysine decarboxylation, indole, methyl red test, Voges-Proskauer, citrate utilization Serological identification of both E. coli and Salmonella were carried out according to Edward and Ewing [14] using slide agglutination technique.

Serotyping of isolated Salmonella serovars strains were carried out in Animal health researches institutes, Dokki, Giza. While E.coli strains were carried out in Department of poultry disease faculty of Veterinary Medicine Cairo University.

PCR identification

Detection of virulence genes was performed by PCR. Primer sequences and PCR conditions used for the study listed in Table (1). PCR performed in Thermal cycler (techno USA). PCR products were separated and visualized by gel electrophoresis in 1.5% agarose in Tris-acetate-EDTA (TAE) buffer at 100 V. And Gel Pilot 100 bp ladder (QIAGEN, USA) was included in each agarose run, accordingly the amplified product.

Table (1).Primer sequences and PCR product used:

Gene	Primer sequence	PCR product	Reference
For salmonella(Stn)	TTG TGT CGC TAT CATGG CAA CC ATT CGT AAC CCG CTC TCGTCC	617 bp	[15]
For E-coli(16srRNA)	CCC CCT GGA CGA AGA CTG AC ACC GCT GGC AAC AAA GGA TA	401bp	[16]

III. Results And Discussion

Table (2) Prevalence of E-coli spp. Isolated from chicks and Adult yards in ostrich farms

Sample	Chicks			Adult		
	No of sample	+ve	%	No of sample	+ve	%
dropping	40	24	60	75	49	65.3
soil	39	21	53.8	71	40	56.3
feed	13	8	61.5	13	9	69.2
water	9	6	66.6	13	10	76.9
Total	101	59	58.4	172	108	62.7

Table (3) Prevalence of salmonella spp. Isolated from chick and Adult yards in ostrich farms

Sample	Chick yards			Adult yards		
	No of sample	+ve	%	No of sample	+ve	%
dropping	40	10	25	75	21	28
soil	39	8	20.5	71	18	25.3
feed	13	2	15.4	13	1	7.1
water	9	1	11	13	2	15.3
Total	101	21	20.8	172	42	24.4

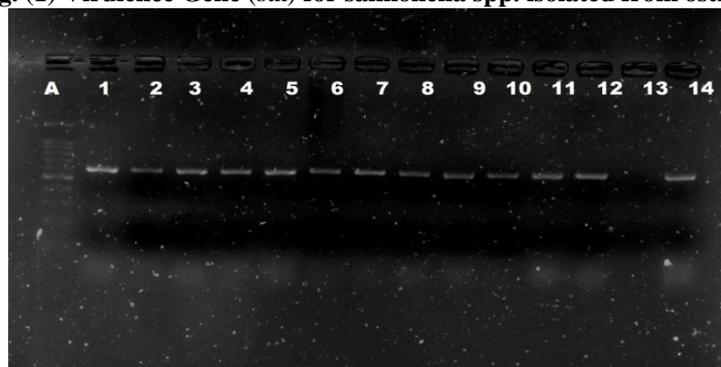
Table (4) Serotyping of E-coli isolated and occurrence of (16s rRNA)

Serotypes		Isolated from	Positive for (16s rRNA)
Isolate	No.		
O44:K74	51	Dropping and water	100% (16srRNA) gene present in all isolated serotype.
O128:K67	37	Dropping , soil, feed and water	
O126:K71	42	Dropping and soil	
O125:K70	25	Soil and water	
Autoagglutination(un typeable)	12	Dropping	
Total	167		

Table(5) Serotyping of salmonella species isolated and occurrence of enterotoxin gene (stn).

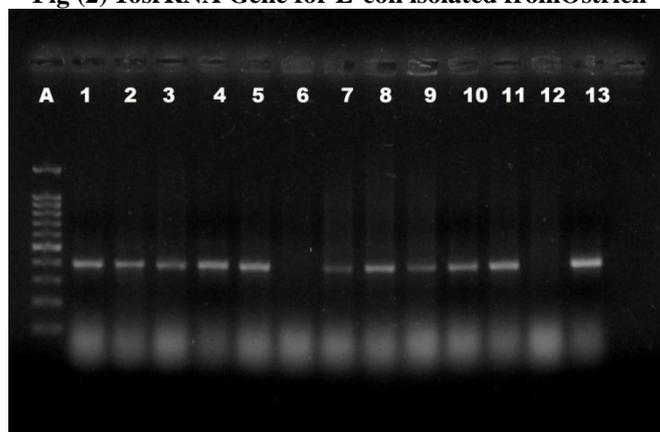
Serotypes		Isolated from	Positive for (stn) gene
isolate	No.		
S. Agama	12	Dropping , feed,	100% (stn) gene present in all isolated serotype.
S. Agona	16	Dropping, soil, egg	
S. wingrove	12	Dropping ,water	
S. Kentucky	19	Dropping , feed	
Autoagglutination(un typeable)	4	feed	
Total	63		

Fig. (1) Virulence Gene (stn) for salmonella spp. isolated from ostrich



Agarose gel electrophoresis showing Salmonella specific PCR of Salmonella isolates using primer set for the stn (617 bp) gene. Lane A: 100- 3000pb DNA ladder; lane 14: Positive control; lane 13.: Negative control; Lane 1 to 12 examined Salmonella.

Fig (2) 16srRNA Gene for E-coli isolated from Ostrich



Agarose gel electrophoresis showing E-coli specific PCR of E-coli isolates using primer set for 16srRNA (401 bp) gene. Lane A: 100- 3000pb DNA ladder; lane 13: Positive control; lane 12: Negative control; Lane 1 to 11 examined Salmonella ; lane 6 :negative sample.

Although little information is known about E-coli and Salmonella spp. in ostriches, the prevention of bacterial contamination in ostrich products and hatching eggs requires detailed knowledge of the main sources associated with its presence in the production system.

The results obtained in (Table 2) clarified that; the overall prevalence of E. coli in all examined samples from chick's yards and adult yards was 58.4 % (59/101) and 62.7% (108 /172) respectively. Nearly similar prevalence result was obtained byNasef,S et al. [17] who found that, the prevalence rate of E. coli in samples collected from ostrich farm (buccal, faecal and nasal swabs, feed, water, liver, and heart) was 40.9% (98/242), while low prevalence rate of E. coli was recorded by [18,19]

The highest percentage of E.coliisolation was obtained from dropping 60% and 65.3% for chicks and adult respectively,followed by soil samples as the percentage was 53% for chicks and 56.3% for adult while the lowest percentage was obtained from feed and water in both chicks and adult ostrich.

Detection of E. coli in feed, water and droppings of rearing ostrich flock may be attributed to the ostrich chicks which may be obtained from infected source with E. coli while The highest prevalence rate (62.7%) of E. coli was detected in environmental samples that collected from breeder flocks over 2 years This may be related to the exposure of breeders' yards to higher level of environmental contamination with dust and wild bird or other animal dropping compared to the environmentally controlled rearing pen.[20]

Metawea Y and Essam S et al. [21, 22] detected E. coli in 22.2, 20.8 and 72.2% of examined feed, water and litter, respectively. While the lowest prevalence rate reported by [23] who found that, the prevalence of E. coli in examined water, feed, and litter samples collected from poultry farms were 14.58, 15, and 27.92%, respectively.

Timur et al. [23]found that, E. coli was detected in 55 out of 135 (40.7%) droppings samples of healthy ostrich. the Lower prevalence rate was recorded byBoci,J et al. [24]who detected E. coli in 0.26% (4 isolates) of examined ostrich droppings[25] found that, the prevalence of E. coli in poultry feed was 57.14%. In the other hand, [26] reported that, the prevalence rate of E. coli in poultry feed was 2.3%, While [27] reported that, the prevalence of E. coli in drinking water of poultry farm was 36.8%. While [28] detected E. coli in 9.14% of examined water samples from poultry farms.

(Table 3) clarified that; the overall prevalence of salmonella spp. in all examined samples from chick's yards and adult yards was 20.8 % (21/101) and 24.4% (42 /172) respectively. This result is nearly agreed withHamedet al. [29] who isolateSalmonella with (28.57%) of (4/14) from ostrich. While disagree withMetaweaY [30]who found salmonella with percentage of 8.6 % (27/315) in ostrich farm.

The results clarified that, the highest prevalence (24.4%) of Salmonella was recovered from environmental samples (feed, water, dropping) that collected from adult breeder flocks followed by that collected from chick flocks (20.8%). These findings may be attributed to the variation in system of housing as the rearing ostrich flock was housed in environmentally controlled pens, while adult breeder flocks were housed in open yards with sandy soil (more liable to environmental contamination with Salmonella) and breeder yards may be fenced by fence to other animal species as large animal or even poultry . The obtained results agree with those reported byBrazil ,Skov, M et al. and Marin,C et al.[31,32,33]proofed that Even with adopting good sanitary management, it is impossible to guarantee that ostriches are not exposed to pathogenic microorganisms, because the farming systems are based on alocation of birds in the open air allowing contact with other animals (wild birds, rodents, insects and others) which increase the capability to transmit Salmonella spp.

The prevalence of Salmonella in examined rearing chick ostrich droppings was 25%(10/40) and the highest prevalence rate 28%(21/75) was recovered from droppings of adult breeder flocks over 2 years old. The highest prevalence rate of Salmonella in adult breeder flocks compared to rearing flock explained bymetawea [30]who report that it may be attributed to old age of animals which able to carry and intermittently shed Salmonellae for an extended period in addition to the behaviour of the ostrich chicks which always pick up faeces of other chicks. Once one chick is infected with Salmonella the infection will be spread rapidly through the flock. These findings are nearly similar to those reported by [34,35]meanwhile Effata and Moursi[36]foundthat,the prevalence of Salmonella in faces, cloacal swabs and internal organs of ostrich flocks over three months at Ismailia province was 9.23%, while Salmonella was not recovered from ostrich flock under 2 months old , Oliveiro et al. [37]found that no Salmonella was recovered from 80 droppings samples from ostriches of different ages at Brazilian southeast region.

The obtained results indicated that, ostrich droppings is one of the most important sources of Salmonella in ostrich farm as there was a positive correlation between the presence of Salmonella in dropping and the prevalence of Salmonella in both feed and water.

We found nearly similar percentage of salmonella in feed for both adult and chicks this may be due to usage of the same feed for both after grinding for chicks in most farms. The exposure of feed of breeders flocks

to higher level of contamination from farm environment (dropping of ostrich and rodents, contaminated sandy soil). also, Salmonella may be derived from contaminated feed components (animal and/or pant origin). Jones F. and Richardson [38] revealed that 8% of examined completed feed from feed mills was contaminated with Salmonella.

From table (3) the prevalence of Salmonella in examined water samples from chick and adult yards were 11% (1/9) and 15.4% (2/13) respectively. The highest prevalence rate was detected in water from breeder flocks that may be attributed to the contamination of tanks and drinkers from the environment (ostrich, wild bird and rodents droppings, and sandy soil), unchanging the water frequently, in addition to the less use of antibiotic in breeder flocks.

Salmonella was found in smaller percentage in chick yards this may be attributed to the using of antibiotics in drinking water during 1-10 days of rearing chicks.

The variations in the prevalence among farms may be attributed to the hygienic measures applied in each farm, system of housing, water source, site of sampling, season, addition of antibiotics and the health status of flock. The results indicated that the drinking water would be another way to introduce Salmonella spp. in ostrich farming.

The data illustrated in (Table 4) clarified that, the serotype of E. coli obtained from positive E. coli sample was O44:K74 (51 strain) O125:K70 (25 strain), O126:K71 (42 strain) O128:K67 (37 strain) and finally Autoagglutination (un typeable) (12 strain). Many researches isolated the same serotypes in addition to more serotypes from ostrich, poultry and their environment, Metawea Y. et al. [19] isolate O128:K67 and O126:K71 and other serotype from ostrich farm, Ali, A and Youssef, E [33] detected E. coli O44, , O125 in ostrich showing respiratory problems.

The data illustrated in (Table 5) clarified that, the serotype of salmonella spp. obtained from positive salmonella sample was S. Agama (12 strain) S. Agona (16 strain), S. wingrove (12 strain) S. Kentucky (19 strain) and finally Autoagglutination (un typeable) (4 strain). Many researches isolated the same serotypes in addition to more serotypes from ostrich, poultry and their environment [30,39]

In Fig. (1) PCR assay carried out for the detection of the *stx* gene in Salmonella isolates and showed that the gene was present in all the isolates (100%) that were demonstrated by the presence of a 617 bp PCR product. These findings are in agreement with Ezzat et al. [40].

In Fig. (2) PCR assay carried out for the detection of the 16S rRNA gene in E. coli isolates and revealed that the gene was present in all the isolates (100%) that were demonstrated by the presence of a 401 bp PCR product. Gehua Wang et al. [10] found that An internal control of E. coli 16S rRNA was present in all of the E. coli samples, thus confirming the presence and the quality of E. coli DNA amplification as well as validating the PCR conditions.

IV. Conclusion

Ostrich are susceptible to a number of infectious agents which are common to other avian species, our work clarified that, ostrich environment play very important role in transmission of these infectious agents especially E. coli and salmonella species, ostrich droppings is one of the most important sources of E. coli and Salmonella in ostrich farm and the prevalence of these pathogenic agents in ostrich environment depend mainly on the degree of the hygienic measures used in each farms.

V. References

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